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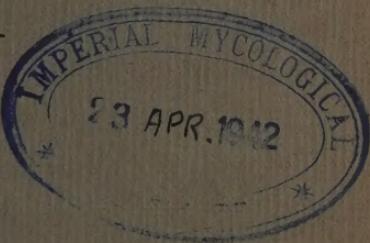
COMMONWEALTH

OF AUSTRALIA



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OF  
THE COUNCIL FOR SCIENTIFIC  
AND  
INDUSTRIAL RESEARCH

NOVEMBER, 1941



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## Studies on Fly Strike in Merino Sheep.

No. 6.—Comparison of the Incidence of Crutch Strike in Plain-Breeched Sheep and in Wrinkly-Breeched Sheep Treated by the Mules Operation.

By I. L. Johnstone, B.V.Sc.\* and N. P. H. Graham, B.V.Sc.\*

### 1. Introduction.

Plain-breeched sheep are not entirely immune from crutch strike, and it is claimed by pastoralists and others that in severe fly waves a relatively high percentage of such sheep may be struck. Because of this, some pastoralists have believed that the Mules operation would have limited use; it was contended that the operation reduced the predisposition to crutch strike of wrinkly-breeched sheep to that of plain-breeched sheep and that, since plain-breeched sheep are commonly struck in severe fly waves, the Mules operation must largely fail under such circumstances. Previous trials of the Mules operation have generally been conducted in wrinkly flocks and have given little information about the incidence of crutch strike among treated sheep compared with that among naturally plain-breeched sheep. Mr. Euston Young, of "Noondoo," Queensland, kindly allowed us to carry out a trial which was designed to give information on this point.

### 2. Experimental Procedure.

Three groups were selected from among some 3,000 flock ewe-weaners. Two days after shearing in June, 1940, they were examined for breech conformation in the standing position in a classing race, and all the plain-breeched sheep, numbering 530, were drafted off. From the remaining more wrinkly portion of the flock, 1,200 sheep were drafted off and then divided at random into two equal groups. To one of these groups the Mules operation was applied and the other group served as a control. These three groups will be referred to as "plain," "treated," and "control."

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\* An officer of the Council's McMaster Animal Health Laboratory, Sydney.

For the Mules operation, the sheep were held on a rail in the lamb-marking position and a single strip of skin was removed from each side of the breech with dagging shears. The skin excision was commenced just above the root of the tail and ended some three or four inches below the vulva. It included the medial breech fold, and its maximum width was about two inches. No additional excisions were made to remove lateral folds. This technique has been used in all recent trials. In earlier trials, roleut secateurs were used, the excisions were usually narrower and commenced at the side of the tail, and if the lateral breech folds were prominent they were removed by separate excisions.

The three groups were given distinctive marks and were mustered periodically for examination. On these occasions the incidence of strike in each group was recorded. It was not practicable to keep records for each sheep. At the first muster in August, 1940, six sheep in the treated group were retreated, and fifteen sheep in the plain group were removed to the control group because slight wrinkling of the crutch was detected.

During the course of the trial there was a decrease in numbers in each group. This was due mainly to sheep escaping from the paddocks and to loss of identification marks. Early in the trial there was an influx of sheep from an adjoining paddock but as these were all of a similar type to the controls they were included with that group. Fluctuations in group numbers at different musterings were mainly due to incomplete mustering.

Towards the end of the trial in May, 1941, the sheep were classed as a single flock, and the percentage of culls derived from each of the three groups was determined.

### 3. Seasonal Conditions During the Trial.

In June and July, 1940, there were no strikes and the sheep were not examined, but with the onset of warmer weather in August blowflies became active again. In spite of a dry spring they continued to strike the sheep till the end of October, but ceased completely during November and December. Feed was scarce and the sheep lost some condition, particularly those in the control group. Over six inches of rain fell in January, 1941, feed came away rapidly and a severe fly wave developed. At this time the sheep were carrying seven months' growth of wool on the crutch. The number of strikes in the control group became unmanageable and the sheep in all the groups were crutched on February 3rd. Blowflies remained active till the trial ceased at the end of May, 1941.

### 4. Results.

The strikes recorded at each muster are set out in Table 1. The strikes were classified, according to their situation, as "true crutch strikes," in which only the inner breech was involved, "tail strikes," in which only the tail was involved, and "strikes of doubtful origin," in which both tail and breech were involved and the strike might have originated in either situation. In Table 1 the columns headed "All Strikes Involving Breech" include "true crutch strikes" and "strikes of doubtful origin."

TABLE 1.—PERCENTAGE OF SHEEP STRUCK AT EACH MUSTERING.

Date.	PLAIN GROUP.				TREATED GROUP.				CONTROL GROUP.			
	Number of Sheep Examined.	Percentage of Sheep Struck.			Number of Sheep Examined.	Percentage of Sheep Struck.			Number of Sheep Examined.	Percentage of Sheep Struck.		
		True Crutch Strikes.	All Strikes Involving Breech.	True Tail Strikes.		True Crutch Strikes.	All Strikes Involving Breech.	True Tail Strikes.		True Crutch Strikes.	All Strikes Involving Breech.	True Tail Strikes.
15.8.40 ..	540	% 1·0	% 1·2	%	590	% *0·3	% 0·3	%	710	% 4·9	% 5·2	% 0·4
11.9.40 ..	497	0·2	0·2	..	574	..	..	0·5	631	8·7	9·5	1·1
3.10.40 ..	435	0·2	0·2	0·2	491	..	..	0·2	555	2·3	2·8	0·5
29.10.40 ..	500	..	0·4	0·2	556	..	..	1·3	613	7·6	8·8	2·6
†3.2.41 ..	427	8·6	13·5	4·9	418	0·7	3·5	8·8	386	31·1	42·0	4·6
4.3.41 ..	446	..	..	..	410	..	..	..	352	3·4	3·9	0·3
4.4.41 ..	436	0·6	0·6	0·4	412	..	..	0·2	521	6·1	8·0	1·0
29.4.41 ..	421	1·4	1·9	0·2	412	0·2	0·2	0·2	354	11·8	17·5	..
14.5.41 ..	440	2·5	2·5	0·4	417	..	0·2	0·2	366	10·1	11·8	0·5

\* Two sheep, one of which required retreatment.

† All groups were crutched on this date.

As may be seen in Table 1, there were many strikes in the control group during the early months of the trial when very few strikes were recorded in the other two groups. Throughout the trial there were appreciably fewer crutch strikes in the treated group than in the plain group. When the fly wave commenced, in January, 1941, there was a rapid increase in true crutch strikes in all groups. Nevertheless, despite the fact that the sheep were carrying seven months' wool at the time and that 31·1 per cent. of the control sheep showed true crutch strikes, only 8·6 per cent. of the plain group and less than 1 per cent. of the treated group were so struck.

The effect of crutching during the fly wave is clearly seen from Table 1. It will be noticed that whereas this single mid-season crutching gave the treated group almost complete protection from crutch strike for some three months, the protection conferred on the plain group was not so durable and a considerable percentage of true crutch strikes was recorded in the control group within a month of crutching.

The incidence of true tail strike was slightly less in the plain group. The Mules operation does not affect the incidence of tail strike, and the plain-breeched sheep would tend to have fewer tail wrinkles than the sheep in the other two groups.

All the sheep in the trial were classed in May, 1941, by a classer who was not aware of the details of the experiment and did not know the meaning of the group identification brands. The groups were then drafted and the culls were taken out. The percentage of culls in each group was as follows: Plain group 26·6, treated group 31·1, control group 48·6.

### 5. Discussion.

The plain-breeched sheep incurred few crutch strikes, even though a severe fly wave occurred when they were in seven months' wool. Nevertheless, throughout the trial there was appreciably less crutch strike in the treated group than in the plain group. Just before the

February crutching, when the effects of the fly wave were most severe, the treated group incurred less than 1 per cent. of true crutch strike compared with over 8 per cent. in the plain group and over 30 per cent. in the controls. In addition, the effect of crutching in controlling crutch strike lasted longer in the treated group than in the plain group.

There may have been some sheep in the plain group with very small breech folds, but this cannot explain the higher strike incidence in this group than in the treated group. Many of the plain-breeched sheep that were struck were carefully examined, and no evidence of breech folds could be detected. It is thought that contraction of the Mules operation wounds may have stretched the skin of the "bare area" surrounding the vulva, and thus reduced the risk of urine staining.

If these sheep had been docked at the length which is now recommended, namely four inches, the incidence of tail strike throughout the groups would probably have been much lower.

The very brief period of protection against crutch strike which crutching gave to the control group was largely due to the difficulty of crutching such sheep cleanly and efficiently.

The results of the classing have considerable practical significance. They demonstrate the effect of fly strike on the general appearance and well-being of the sheep. In this instance 17 per cent. more sheep were culled from the controls than from the treated sheep. As these two groups were selected at random from the same lot of wrinkly-breeched weaners and had grazed together throughout the trial, the additional 17 per cent. culled from the controls can be attributed to the effects of fly strike in that group. The classer stated subsequently that the removal of the central breech folds by the Mules operation had not noticeably "disguised" the breech conformation. He could not have been influenced by it in classing because the groups were mixed and he did not know which sheep had been treated. Thus, crutch strikes which could have been prevented by the Mules operation were responsible for the rejection by culling of 17 per cent. of the control group. Only 5 per cent. more sheep were culled from the treated group than from the plain group.

## 6. Conclusions.

- Plain-breeched sheep selected from a relatively wrinkly flock incurred more crutch strikes than wrinkly-breeched sheep on which the Mules operation was performed.
- Crutching gave more effective and lasting protection against crutch strike in plain-breeched sheep and in wrinkly-breeched sheep treated by the Mules operation than in untreated wrinkly-breeched sheep. This is largely due to the difficulty of crutching wrinkly sheep efficiently.
- The Mules operation and a mid-season crutching almost entirely prevented crutch strikes in wrinkly-breeched sheep from one shearing to another, despite a severe and prolonged fly wave.
- The effect of fly strike on the general health of the sheep led to a high rate of culling which could have been avoided by proper use of the Mules operation.

# Fly Strike Investigations—Experimental Studies on the Surgical Removal of Breech Folds in Lambs.

## The Mules Operation at "Marking-Time."

By N. P. H. Graham, B.V.Sc.,\* J. H. Riches, Ph.D., B.Agr.Sc.,† and  
I. L. Johnstone, B.V.Sc.\*

### Introduction.

Mules (1935) enunciated the principle of the removal from ewes of the medial breech folds which were subject to wetting by urine and which, as a result of moisture and of the scalding produced, became attractive to the primary blowfly, *Lucilia cuprina*. The Mules operation has been investigated by several workers (Seddon, 1935; Beveridge, 1935; Belschner and Hindmarsh, 1937; and Gill and Graham, 1938, 1939, 1940). They have dealt with the results obtained by carrying out the operation on weaners or older sheep.

Only one trial, recorded by Mackerras (1935, 1937), has dealt with the operation on lambs at marking-time. The trial reported was carried out by the Australian Pastoral Company at "Noondoo," Queensland, and when the lambs were examined some three weeks after the operation the results appeared to be quite satisfactory. Subsequent to the published report, however, the treated lambs became similar in breech conformation to a group of untreated lambs left as controls and suffered to approximately the same extent from fly strike. The failure of the operation in this instance was possibly due, in part at least, to the removal of insufficient skin.

As marking-time is usually the most convenient time to apply the Mules operation, and as highly successful results are obtained by its use at weaning, it was desirable to investigate further its use at marking-time.

Before marking-time can be considered a suitable time to carry out the operation, it is necessary to know: (a) if the operation at this time, i.e. on young lambs, gives effective and permanent results; (b) if it can be done quickly enough to avoid undue delay in the marking procedure; and (c) if it is necessary to treat all lambs or only those that show obvious medial breech folds. In the three field trials described in this paper, particular attention has been paid to these points.

### Experiment I.

The first experiment, which may be regarded as preliminary, was carried out at "Gilruth Plains" by one of us (J.H.R.) at lamb-marking in November, 1939, to test: (a) the practicability and efficiency of the operation performed at marking time, and (b) the supplementary effect of longer docked tails, which had been shown previously by Gill

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† Officer-in-Charge, National Field Station, "Gilruth Plains."

and Graham (1939) to give some measure of protection against breech strike. Separate investigation of the effect of tail length has been made, and the results have been reported elsewhere (Riches, 1941).

Owing to the extremely dry conditions that prevailed during 1940, too few fly strikes occurred to give measurable differences between the groups. The results were therefore measured during this period by the effect of the operation on breech conformation which numerous observations had shown to provide a measure of predisposition to breech strike. During the early months of 1941, however, a fly wave occurred, and strike records are quoted for that period.

#### *Procedure.*

The selection of treatments of the groups was decided by randomization, and eight marking cradles were used. The lambs were classified according to breech conformation while in the cradles, but since the posture enforced by the cradles tends to obliterate the skin folds by tension, the looseness of the skin on the crutch, as judged by mammal examination, was also taken into consideration. The large number involved was relied on to give an approximately even distribution of A, B, and C class lambs in the groups.

In all, 762 lambs were included in the treated group. The operation was performed, irrespective of classification, on all but twenty which were considered too plain to warrant surgical interference. *It was performed by commencing the cut at a point about level with the upper commissure and continuing it some distance below the tip of the vulva (cf. Experiment 2, p. 236).* The lower limit of the cut differed according to the wrinkliness of the lamb; in big C class individuals the cut terminated about two inches below the vulvar tip. The operation was performed by the same operator throughout.

#### *Results.*

The classifications at marking in November, 1939, and after shearing in April, 1940, are given in Table 1.

TABLE 1.—EFFECT OF THE OPERATION AS PERFORMED IN EXPERIMENT 1 ON SUBSEQUENT BREECH CONFORMATION.

	Treated.				Controls.					
	A.	B.	C.	Total	A.	B.	C.	Total		
<hr/>										
November, 1939—										
Classified in cradles	..	..	67	461	43	571	65	447	59	571
<hr/>										
April, 1940—										
Classified standing	..	..	263	249	19	531	85	350	102	537

The differences in numbers examined on the first and second classifications are due to loss of identification tags, short mustering, and deaths. The numbers of sheep in the treated and control groups at weaning in April were approximately the same. It is concluded, therefore, that the treatment had not, of itself, led to losses by death.

Nor did it cause appreciable loss of condition in the sheep, for the mean body weights of two groups of 150 sheep from the treated and control groups were not significantly different when they were weighed some five months after the experiment began.

The November classification shows that the groups were reasonably similar with regard to breech conformation when the experiment was commenced but, on account of the difficulty of classifying the lambs in the cradles, reliance cannot be placed on these figures for comparison with the April classification.

The April classification shows that the operation had a considerable effect on breech conformation. As the treated group included 249 B class and 19 C class lambs, the result left much to be desired. However, a large number in the treated group were placed in the B class because a small piece of fold remained near the butt of the tail producing a condition entirely artificial and one which strictly does not fit into the accepted classification according to susceptibility to strike.

The year 1940 was extremely dry and few strikes occurred. Heavy rains fell early in 1941 and a "fly wave" soon occurred. The sheep were crutched on 29th January, 1941, and the numbers of strikes then recorded are shown in Table 2.

TABLE 2.—STRIKES RECORDED AT CRUTCHING IN JANUARY, 1941  
(EXPT. 1).

Group.	Number of Sheep.	Number of Strikes.	Strikes per 100 Sheep.
Treated .. .. ..	479	95	19.8
Controls .. .. ..	487	116	23.8

Examination of these sheep after crutching showed that the operation had failed to modify the breech conformation sufficiently, and, in an endeavour to rectify this, 94 per cent. of the sheep in the treated group were retreated on 11th February, 1941.

Flies, particularly the bush fly (*M. vetustissima*), were very prevalent at the time of retreatment and some strikes occurred in the operation wounds, but they are disregarded in this consideration. Further records of strike incidence for comparison of the two groups were made between 27th March and 19th May, just prior to shearing, and are shown in Table 3.

TABLE 3.—STRIKES OCCURRING SUBSEQUENT TO RETREATMENT  
(EXPT. 1).

Group.	Number of Sheep.	Number of Strikes.	Strikes per 100 Sheep.
Treated .. .. ..	495	35	7.1
Controls .. .. ..	481	235	48.9

Comparison of Tables 2 and 3 shows that although the strike incidence in the control group during April and May was double that prior to the January crutching, the incidence in the treated group after retreatment dropped from 19·8 to 7·1 strikes per 100 sheep, a reduction of 64 per cent.

### Experiment 2.

The second experiment was carried out by two of us (N.P.H.G. and I.L.J.). Again all ewe lambs except those that were entirely plain-breeched were operated on. By re-examining both the treated and untreated lambs after the weaner crutching, it was thus possible to determine: (a) the percentage of treated lambs needing retreatment, and (b) the extent to which lambs that appeared plain on the marking rail had deceived the operator or had developed wrinkles in the meanwhile. Moreover, if a considerable proportion of the plain, untreated lambs appeared wrinkly at weaning time, whereas the treated lambs were plain, it would indicate that the plainness in the treated lambs was due to the operation and not to nutritional or other circumstances. Thus in judging results consideration would be given to both breech conformation of the sheep and the incidence of crutch strike.

An opportunity to carry out this trial was kindly provided by Mr. Euston Young, of "Noondoo," Queensland. The operation was performed in May, 1940, on lambs from a few days to about two months old, and the groups were re-examined after being shorn prior to weaning in September, 1940. Most of the lambs were in good condition at the time of operation, though a few, which had probably lost their mothers, were poor.

#### *Procedure.*

The operation was carried out on the ewe lambs by a single operator, who led the marking team, being followed by a man ear-marking and another who did the tailing and castrating. The operation did not seriously interfere with the speed of marking. About 300 lambs were marked per hour, and from 100 to 120 of these were treated. No special hold was used as it was considered easier for the operator to adapt himself to the usual procedure than for the catchers to hold the lamb in an unusual position. In this trial only those lambs were treated that were considered to be wrinkly or to have loose skin on the breech, the operator judging the necessity or otherwise for treatment while the lambs were held on the rail. When no wrinkles were visible the skin was pulled on the crutch to see if it was loose. *A single strip of skin was removed from the treated lamb starting about 1 inch above the butt of the tail and continuing well below the vulva. The width of the strip varied from about  $\frac{3}{4}$  to 2 inches, according to the degree of "development."* This was a more radical operation than that used in Experiment 1.

In all, 1,496 ewe lambs were treated, and 525, which were judged to be plain, were left untreated.

#### *Results.*

(a) *Control ("plain"-breeched) Group.*—When these lambs were examined off shears, some 15 weeks after the commencement of the experiment, 363 of the original 525 were classified according to breech

development. It was not practicable to examine the entire group as shearing was in progress at the time. The proportion of A, B, and C class weaners in this group, and of those showing evidence of having been struck during the period between marking and weaning, are shown in Table 4.

TABLE 4.—CLASSIFICATION AND STRIKE INCIDENCE AMONG UNTREATED WEANERS CONSIDERED PLAIN-BREECHED AT MARKING-TIME—(EXPT. 2).

Classification.	Number (Per cent.).	Number Struck.	Per cent. Struck.
A .. .. .. ..	95 (26.5)	1	1.0
B .. .. .. ..	230 (63.0)	55	24.0
C .. .. .. ..	38 (10.5)	21	55.0
Total .. .. .. ..	363	77	21.0

As these sheep were all regarded as A class at marking-time a change in conformation had apparently occurred. However, at marking-time the lambs were examined when held on the rail with the legs stretched in the marking position, whereas after shearing they were examined in the normal standing position. The position and the presence of relatively long wool on the breech of the older lambs at marking-time would account largely for the apparent discrepancy in classification. Moreover, although the season had been dry, the ewes had nourished the lambs well and under such conditions an increase in "development" may have taken place during the suckling period.

(b) *Treated Group.*—Of the 1,496 lambs that were treated at marking-time, 1,399 were re-examined after shearing. The lambs were also examined for evidence of fly strike between marking and weaning.

Only 107, or 7 per cent., were considered to require retreatment, but as the experimental sheep were to be returned to the main flock this was not actually carried out.

The incidence of fly strike in the treated group was low, 55, or 4 per cent., having been struck, and most of these were among the animals requiring retreatment.

The incidence of fly strike in the treated and untreated groups is summarized in Table 5.

TABLE 5.—INCIDENCE OF FLY STRIKE IN TREATED AND UNTREATED GROUPS.—(EXPT. 2.)

Group.	Number of Sheep in Group.	Number of Sheep Struck.	Per cent. Struck.
"Plain" lambs, untreated ..	363	77	21
Lambs requiring retreatment ..	107	22	20
Successfully treated lambs ..	1,292	33	2.5
	1,399	55	3.9

Some 10 months after the operation had been performed, an opportunity occurred to re-examine 1,185 of the treated sheep. Only 97, or 8.5 per cent., were considered to require retreatment. Thus little, if any, change in breech conformation had occurred since weaning time, 6½ months earlier. This is of particular interest because heavy rain had produced an abundance of feed during the three months preceding the re-examination.

It was not practicable to record strikes among the sheep on this occasion.

### Experiment 3.

The Mules operation was performed by two of us (J.H.R. and I.L.J.) on half the 1940 drop of lambs at "Gilruth Plains," the other half being kept as controls. Further information was also sought on the effect of long and short docking. The "long" tail was 4 inches long after docking. The treated and control groups were, therefore, subdivided giving four groups as follows:—Docked-long plus the Mules operation, docked-short plus the Mules operation, controls docked-long, controls docked-short.

#### Procedure.

No attempt was made to pick out the plain sheep. The site and extent of the skin excision was as described in Experiment 2. Of the 506 treated sheep, 272 were ewes and 234 were wethers.

Very dry conditions were experienced between October, 1940, when the lambs were marked, and January, 1941, and no strikes were recorded over this period. Losses from drought were heavy and the number which had been treated by the Mules operation was reduced to 389. These sheep were crutched in February, 1941, re-examined and, where necessary, retreatments were carried out. Among the ewe lambs 21, or 10 per cent., required retreatment, and among the wethers, 7 or 3.9 per cent. Although the proportion of ewe lambs retreated was 10 per cent., many of them required the removal of only a small piece of skin from one side.

In Table 6 is given the incidence of fly strike in the ewe portion of the flock. Up to 31st May, 1941, no strike occurred among the wethers, in spite of a severe fly wave during the autumn.

TABLE 6.—STRIKE INCIDENCE IN EWE LAMBS BETWEEN 1/1/41 AND 31/5/41.—(EXPT. 3).

Group.		Number of Sheep.	Number of Strikes.	Strikes per 100 Sheep.
Mules Operation, "Long" tail	..	114 } 222	1 } 10	0.9 } 4.5
Mules Operation, Short tail	..	108 }	9 }	8.3 }
Control, "Long" tail	..	110 } 215	23 } 82	20.9 } 38.1
Control, Short tail	..	105 }	59 }	56.2 }

These results are very similar to those obtained in Experiment 2. The extremely low incidence of strike in the "long tail plus Mules operation" sub-group, viz., less than 1 strike per 100 sheep, is remarkable in view of the fact that fly-strike was particularly prevalent at the time and 56.2 strikes per 100 sheep occurred in the control group with the short-tail.

### Discussion.

The failure of the early trial reported by Mackerras (1935, 1937), and to some extent of Experiment 1 in the present series, might have been due to post-operative growth of skin and further breech development in the treated lambs, but as in both these trials a relatively small amount of skin was removed, this is believed to be the main cause of the failure. There is now tangible evidence (Carter, 1941) that development is greatly influenced by the plane of nutrition, being much more pronounced in rapidly growing sheep on a high plane than in slowly growing sheep on a low plane of nutrition. Experiments 2 and 3, however, show that, provided sufficient skin is removed, the effects of the operation are not nullified by nutritional factors. The lambs in Experiment 2 were well grown and remained on a relatively good plane of nutrition throughout. The lambs in Experiment 3 were born during one of the worst droughts the district has experienced and conditions did not improve for 8 to 10 weeks after the operation was performed. Then heavy rains resulted in abundant feed. This continued till the end of the trial, a period of some five months during which the lambs grew and thrived. Observers who were associated with all these trials state that in Experiment 2 approximately four times as much skin was removed as in the early trial recorded by Mackerras, and about twice as much as in Experiment 1 of the present series.

As a result of the more extensive excision of skin, there was a decrease in the percentage of lambs requiring retreatment to about 8 per cent. in Experiment 2, and 10 per cent. in Experiment 3, and it would seem that a further reduction is unlikely as a result of any modification of technique.

It is reasonable to conclude that inadequate treatment is the main cause of failure and that, if properly carried out, the Mules operation performed on lambs at marking-time will give effective and permanent results. The similar and highly satisfactory results in Experiments 2 and 3 were obtained by different operators on different flocks in different localities and under conditions which were dissimilar.

Lambs were found to be more difficult to treat efficiently than freshly crutched weaners. The small size of the folds in some lambs may make it difficult to hold them without some risk of cutting the fingers. The wool on the crutch of the older lambs was frequently from 1 to  $1\frac{1}{2}$  inches long, especially in those with a hairy birth coat, and this masked the distribution of the folds. In such cases, it was difficult to determine whether the shears were cutting skin or wool, so that the actual skin excision was sometimes less extensive than was intended.

It may be possible to obtain a somewhat higher degree of efficiency with experience; none of us had operated on lambs prior to these trials, though we had had experience with weaners.

The operation need not delay the marking routine, especially if marking is done with the care that its importance warrants.

In Experiment 2 and, to a lesser extent, in Experiment 1, attempts were made to differentiate between plain and wrinkly lambs on the marking-rail with a view to leaving the former untreated. In both cases it was a failure, as a very high proportion of these "plain" sheep were found to have B or even C class breeches when they were examined some months later. Hence, if the Mules operation is to be performed at marking-time all lambs should be treated, irrespective of the appearance of the breech when the animal is held on the marking-rail.

### **Conclusions.**

1. Provided the operation is carried out as described under Experiment 2, the percentage of lambs requiring retreatment will be low and the protection afforded against crutch strike will be high.

2. The position in which the lamb is held for marking, coupled with the presence of wool on the crutch, make it difficult to detect with certainty those plain-breeched lambs which could be omitted from the operation. The use of lamb-marking cradles increases this difficulty. Hence, where the Mules operation is applied at marking-time, all ewe lambs should be treated.

3. An additional reason for treating all lambs is that some of those that appear plain at marking-time may be found to have B or C class breeches as weaners, some development apparently having taken place in the meanwhile. There was, however, no evidence to suggest that subsequent development had occurred in those lambs which had been adequately treated.

4. With proper organization and a reasonable speed of marking, the Mules operation can be efficiently performed without unduly hindering the proceedings.

5. There was no evidence in these experiments that the lambs were adversely affected by the operation.

6. Although the fly strike incidence among lambs between marking and weaning is generally too low to warrant special control measures, it becomes a serious problem in most areas during wet seasons. The application of the Mules operation at marking-time should provide a large measure of control in such seasons, and since the operation is now advocated for general use in predisposed flocks, the earlier it can be successfully applied the greater will be the benefit derived from it.

### **Acknowledgments.**

We wish to thank Mr. E. Young of "Noondoo" for making the sheep available for Experiment 2, and the members of his staff, particularly Mr. Watson, who assisted with the work.

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## The Mules Operation: Factors to Consider in Selecting Time of Year and Age of Sheep at which to Operate.

By N. P. H. Graham, B.V.Sc.\*

### 1. Introduction.

The protection against crutch strike afforded by the Mules operation has been amply demonstrated, and the interest of sheep-owners throughout Australia has greatly increased since the results of field trials have become more widely known.

The time of year at which to operate and the age of the sheep at which the operation can best be applied depend largely on local circumstances. It is the purpose of this article to discuss the factors concerned and the objects to be attained.

### 2. The Time of Year.

Fresh wounds are relatively unattractive to blowflies. The Mules operation has sometimes been performed at times when many strikes were occurring in the flock, yet few of the operation wounds were struck. Nevertheless, it would be foolhardy to operate when flies are active. If the healing of the wounds is delayed and they become infected and purulent, blowflies are attracted to lay eggs on them, and the moist surface provides food enabling the larvae to develop and establish a strike. This effect may be brought about also by the use of dressings that are irritant or that delay healing for any reason.

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The operation should never be performed when the small bush fly (*Musca vetustissima*) is prevalent. These flies settle in swarms on any wound, causing great irritation and delaying healing considerably. The wounds become seriously infected and many strikes result. In addition, some sheep may die from infection. In others, the infection from the wound localizes in joints causing pus formation and severe lameness.

Little is known of the life history and habits of the bush flies, but they usually disappear rather suddenly with the onset of colder weather in late autumn or early winter and are not seen in appreciable numbers again until the following summer.

The time selected for the Mules operation, therefore, should be (a) after the disappearance of the bush flies in early winter and before the spring blowfly wave, or (b) in the early summer, when the spring blowfly wave is over and the bush flies have not yet become troublesome.

### 3. The Age of the Sheep.

The Mules operation can be performed on sheep of any age and the effect is permanent. Hence the earlier it is performed the longer will be the period of protection and of economic benefit.

The sheep owner may wish to operate on lambs at marking-time, or on recently crutched weaners or older sheep.

#### (a) *The Mules Operation in Conjunction with Lamb-marking.*

At marking-time, all the lambs are caught and held on the marking rail. This is a convenient position for the Mules operation and if it can be applied at that time the additional labour of putting the sheep over the rail on a subsequent occasion is obviated. Graham, Riches, and Johnstone (1941)\* showed that the Mules operation could be applied successfully at marking-time without seriously hindering the marking routine. There were no ill effects from the operation and the wounds healed more quickly than in older sheep.

More experience and practice are needed to operate successfully on lambs than on older sheep. This is mainly due to the small and varying size of the lambs, and to the presence of wool on the breech masking the skin folds. In older lambs, especially those with hairy birth-coats, the wool on the breech may be  $1\frac{1}{2}$  inches long at marking-time, and it is difficult to know whether the shears are cutting skin or wool, so that the skin excision is often smaller than was intended.

In the experience of some pastoralists, heavy losses result from flystrike among lambs between marking and weaning. Others maintain that the incidence of flystrike among young lambs is not sufficient to call for any special preventive measure such as the Mules operation.

In an attempt to obtain definite evidence on this aspect of the blowfly problem, a questionnaire was sent to 30 pastoralists in Queensland, New South Wales, and South Australia, and 28 replies were received. The information they gave may be summarized as follows:—

Broadly speaking, the incidence of flystrike in lambs depends on the time of lambing in relation to the usual spring and autumn "flywaves."

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\* Graham, N. P. H., Riches, J. H., and Johnstone, I. L. (1941).—This Journal, p. 233.

There is more flystrike among lambs in north-western New South Wales and western Queensland than in the cooler areas. However, 22 of the 28 replies stated that few strikes, from nil to 5 per cent., occurred among lambs between marking and weaning except when blowflies were unusually active. The other six replies gave figures ranging from 10 to 30 per cent. under usual conditions and 20 to 80 per cent. in exceptional seasons. These figures were all estimates and were based on the numbers of lambs requiring treatment. Of the 28 replies, seventeen stated that no special treatment of the lambs was warranted because the mustering and handling might involve greater losses from mismothering than would otherwise occur from flystrike. Five replies stated that special measures, such as jetting or the Mules operation, would be warranted when the strike incidence among lambs was unusually high.

In our experience strikes are more frequent in lambs between marking and weaning than is generally realized. At "Noondoo," in south-western Queensland, under average seasonal conditions, we found that 20 per cent. of lambs examined at the weaner crutching had been struck since marking-time. Few, if any, of these strikes had been detected and dressed at the time of their occurrence. Moreover, seasons of unusual fly activity cannot be foretold sufficiently in advance of their occurrence for special preventive measures to be adopted. To deal with a wave of flystrike among suckling lambs by paddock dressing, or mustering for dressing in the yards, is extremely difficult. Young lambs are severely affected, losses from mismothering may be heavy, and the application of the Mules operation at marking-time solves what may otherwise be a difficult and costly problem.

We believe, therefore, that the Mules operation should be employed in conjunction with marking, unless there is some special reason for avoiding its use at that time, e.g., the prevalence of blowflies or bush flies. Labour is thereby saved and a large measure of insurance is obtained against the occurrence of many strikes among the suckling lambs. Moreover, in our experience, strikes are more frequent among lambs than is generally supposed and these strikes will serve to maintain the blowfly population.

#### *(b) The Mules Operation at Weaning Time.*

Weaners are the easiest sheep to treat. The folds of skin on the mid-crutch are more clearly defined. On many properties it is customary to crutch or shear the lambs at weaning-time, and the Mules operation can be performed by a separate team before the sheep are returned to the paddocks. Otherwise a special crutching is necessary before the operation can be performed. Inexperienced operators should practice on weaners until they are thoroughly proficient before applying the operation to lambs at marking-time.

#### *(c) The Mules Operation in Older Sheep.*

If each drop of lambs is dealt with at marking or weaning it will be some years before all the ewes in the flock are treated. Moreover, untreated breeding ewes may be purchased. The application of the Mules operation to older sheep, therefore, needs consideration. The operation itself offers no difficulty provided the ewes have been crutched or shorn within the previous few weeks. Holding and controlling them

in a suitable position for the operation is troublesome owing to their size and weight, unless the number to be dealt with is small. Mr. Young of "Noondoo," Queensland, whose staff has recently operated on some 30,000 ewes of all ages, finds that the ewes can be held more easily on the floor of the wool shed at the exit to a counting out pen, the operator standing outside the shed. Mr. Young has also devised a special box, or cradle, in which ewes can be held for the Mules operation. This apparatus is effective in practice. It has been patented to avoid its exploitation and a description will be published later.

Whatever method may be used for holding the ewes, it involves considerable labour and, as ewes tend to become plainer and less susceptible to flystrike with increasing age, the operation need only be applied to the susceptible sheep.

Ewes that are susceptible to crutch strike may be selected in either of two ways. The first, which is probably the more reliable, is to examine all the ewes after crutching and to draft off for treatment those that show crutch wrinkles. The other method is to mark or brand the ewes that are struck so that they can be run off and treated later, when the strikes have healed and flies are less active. This procedure should also be used to detect sheep of any age in which the operation has not been successful and which need retreatment.

#### 4. Conclusions.

To sum up, beginners should perform the Mules operation on freshly crutched or shorn weaners until they are thoroughly proficient. Subsequently the operation should be performed in conjunction with lamb-marking unless the prevalence of bush flies or blowflies at that time warrants its postponement until the lambs are weaned. Susceptible adult sheep should also be treated and the Mules operation should be applied to any sheep affected by crutch strike, whether previously treated or not, if examination shows that the operation is required.

# A Further Study of Temperature Changes during the "Turning" of Bulk Wheat.

By F. J. Gay, B.Sc., D.I.C.\*

## *Summary.*

A further study of the effects of the turning process on the temperature of bulk wheat has been made, this time under winter conditions.

The mean temperature of the wheat was lowered by 8.9°C. (16°F.). Thus in contrast to the results of the previous study carried out in January, 1941, a marked degree of cooling was achieved.

## 1. Introduction.

In a recent issue of this *Journal* (Gay, 1941)† an account was given of the temperature changes in bulk wheat that was turned in late summer. It was felt at the time that further investigation was needed to determine the best results that could be obtained by this process under Australian conditions. Accordingly, when the opportunity arose to conduct a second study under winter conditions, it was undertaken without delay.

Through the courtesy of Mr. L. S. Harrison, Manager of the N.S.W. Government Grain Elevators, this work was carried out at Temora, where a 50,000-bushel bin of 1940-41 wheat showing marked heating, and the services of the silo operators, were placed at our disposal.

The silo at Temora is similar in type to that at Old Junee which has been described in the previous report. The methods employed at Old Junee for obtaining the temperature readings were used in the present study and the turn made into an adjacent bin as at Old Junee. The location of the two bins at Temora was identical with that at Old Junee, so that maximum exposure on the conveyor belts was impossible.

Temperature and humidity readings were taken (with a sling psychrometer) during the turn (*a*) out of doors, (*b*) in the conveyor tunnel near the open valve, and (*c*) alongside the gallery conveyor above the bins. In addition, after the first turn was completed, three maximum and minimum thermometers were suspended about two feet above the surface of the "turned" wheat in the bin, to check the conditions to which the wheat was exposed in the interval between the two turns.

The first turn from bin No. 16 into bin No. 17 was begun at 1.55 p.m. on 20th August, 1941. Readings were taken regularly until 4.50 p.m. when the flow was stopped. In this time, approximately 7,500 bushels of wheat had passed into the adjacent empty bin. The second turn from bin No. 17 into one of the small working-house bins was begun at 9 a.m. on 21st August, 1941, and lasted until 12.30 p.m. The flow from the first valve lasted for approximately an hour and three-quarters, from the second valve for three-quarters of an hour, and

\* An officer of the Division of Economic Entomology.

† Gay, F. J. (1941).—*J. Coun. Sci. Ind. Res. (Aust.)*, 14: 111-116.

from the third and fourth valves for 15 to 20 minutes each. During the second turn, the flow had to be reduced to enable the silo operators to carry out other duties. This reduced flow, at four-fifths of the normal speed, lasted from 10·15 a.m. to 11·30 a.m. and allowance has been made for this in the analysis of the results.

## 2. Results.

### 1st Turn—

The atmospheric conditions during this turn were as follow:—

- (a) Out of doors at 3·25 p.m., temp. 18·3°C. (65°F.), R.H. 44 per cent.
- (b) Alongside open valve at 2·55 p.m., 17·8°C. (64°F.), R.H. 70 per cent.
- (c) Alongside tripper on gallery conveyor at 4·30 p.m., 23·3°C. (74°F.), R.H. 40 per cent.

Thus, as will be seen from the following paragraph, the temperature of the air to which the wheat was exposed during the turning process was 17° to 22°C. (approximately 30° to 40°F.) below that of the hottest wheat and 2° to 3°C. (approximately 3·5° to 5·5°F.) above or below that of the cooler runs.

During this turn, 960 readings of the temperature of the wheat were obtained; these showed a range of 19°C. (34·2°F.) from 21°C. (69·8°F.) to 40°C. (104°F.). The mean temperature of the wheat during this turn was 35·3°C. (95·5°F.).

The frequency distribution of the temperature readings during the first turn is shown in the upper histogram of Fig. 1.

### 2nd Turn—

The maxima and minima readings of the air temperature above the wheat during the sixteen hours' interval between the two turns were as follow:—

- (a) Near bin walls (i) max. 20°C. (68°F.)  
min. 9·4°C. (49°F.)  
(ii) max. 17·8°C. (64°F.)  
min. 12·8°C. (55°F.)
- (b) Centre of bin max. 20·6°C. (69°F.)  
min. 10°C. (50°F.)

During this turn, 1,110 readings of the temperature of the wheat were obtained; these showed a range of 15°C. (27°F.) from 17°C. (62·6°F.) to 32°C. (89·6°F.). The mean temperature of the wheat during this turn, making allowance for the slow run wheat, was determined from the formula:

$$M = \frac{T_1 + 4/5 T_2}{N_1 + 4/5 N_2}$$

where  $M$  is the mean temperature,  $T_1$  the total (in degrees) of the temperature observations excluding the slow run group,  $N_1$  the number of such observations,  $T_2$  is the total (in degrees) of the temperature observations in the slow run, and  $N_2$  the number of such observations. This mean temperature was 26·4°C. (79·5°F.).

The frequency distribution of temperature readings during this turn is shown in the lower histogram in Fig. 1. This distribution was obtained by removing from the total distribution of all readings one-fifth of the readings during the slow run. As there were 1,110 readings in all, and 420 in the slow run, the adjusted distribution histogram is based on 1,026 readings.

The effects of the turning process were, therefore, a lowering of the mean temperature of the wheat by  $8.9^{\circ}\text{C}$ . ( $16^{\circ}\text{F}$ .), and a narrowing of the range of temperature by  $4^{\circ}\text{C}$ . ( $7.2^{\circ}\text{F}$ .). Comparison of the two histograms in Fig. 1 shows that the readings falling in the four highest

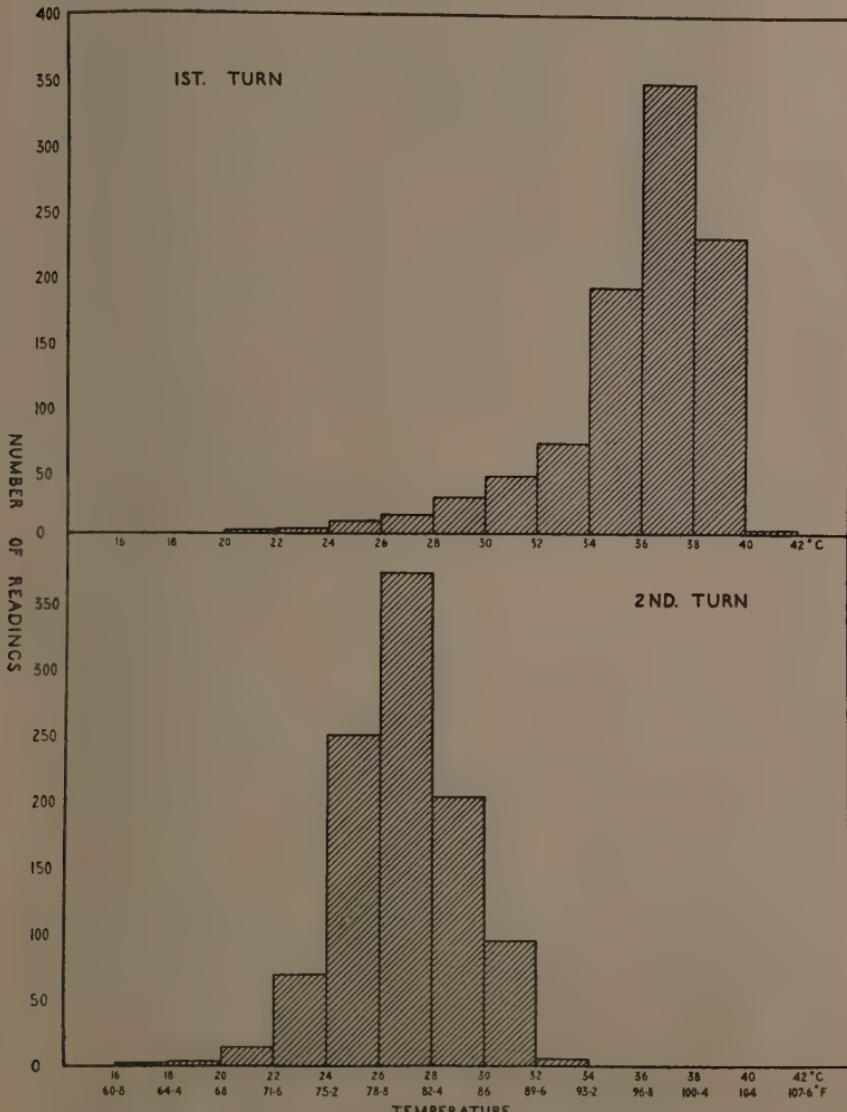


FIG. 1.—Histograms showing, for each of the two turns studied, the distribution of readings over the range of temperature recorded.

temperature distribution classes have disappeared as a result of the turning. In the first series of readings 815, or 85 per cent., were of  $33^{\circ}\text{C}$ . ( $91\cdot4^{\circ}\text{F}$ .) or over, whereas in the second series, not one reading of as much as  $33^{\circ}\text{C}$ . was recorded.

### 3. Discussion.

This study shows that turning small parcels of wheat (up to 7,500 bushels) under winter conditions can bring about a marked reduction in the mean temperature of the wheat. In other words, true cooling takes place in contrast with the averaging effect which occurs when wheat is turned in the summer. In the previous study at Old Junee in January, 1941, in which the mean temperature of the wheat was lowered only  $1\cdot2^{\circ}\text{C}$ . ( $2\cdot2^{\circ}\text{F}$ .), the most important result was the breaking up of pockets showing dangerous extremes of temperature.

Several factors have contributed to this difference. Firstly, the mean temperature of the hot wheat was almost  $4^{\circ}\text{C}$ . (approximately  $7^{\circ}\text{F}$ .) higher at Temora than at Old Junee. Secondly, the air temperature to which the wheat was exposed during turning was lower at Temora. Consequently, a greater degree of cooling could be expected as a result of this increased temperature differential. Thirdly, the greater part of the walls of the empty bins into which the wheat was turned would have a temperature well below that of the mean of the wheat (the mean wall temperature was probably not in excess of  $17^{\circ}$  to  $18^{\circ}\text{C}$ . ( $62^{\circ}$  to  $64^{\circ}\text{F}$ .)), and this would result in a lowering of the temperature of the wheat in contact with the walls. Lastly, the temperatures recorded overnight above the surface of the wheat were  $14^{\circ}$  to  $26^{\circ}\text{C}$ . ( $23^{\circ}$  to  $47^{\circ}\text{F}$ .) below that of the mean temperature of the wheat. Neglecting the fact that wheat forms a conical heap when turned into a bin, the surface area of wheat exposed to this cold air was in excess of 1,000 square feet, so that a considerable heat transfer from the wheat to the air above could take place.

Obviously, if the mean temperature of the wheat is relatively constant, similar bulks of wheat can be expected to experience a greater heat loss to the bin walls and surrounding air in winter than in summer. This effect, however, would tend to diminish as the quantity of wheat turned was increased, since the surface exposed to walls and air does not increase proportionally with the volume. Accordingly, it would be unwise to expect to achieve a reduction in mean temperature, similar to that described above, by turning a full 50,000-bushel bin of hot wheat under similar conditions.

### 4. Acknowledgment.

It is a pleasure to acknowledge the assistance given by Mr. R. N. McCulloch, of the N.S.W. Department of Agriculture, and Mr. D. Gilmour, of this Division, in making the numerous temperature readings in this study.

# Red Clover Seed Production at Moss Vale, N.S.W.

By K. Loftus Hills, B.Agr.Sc.\*

## 1. Introduction.

It has been assumed that the reliable commercial production of red clover seed in Australia is impossible owing to the absence of humble bees (*Bombus spp.*), which are the recognized pollinating agents for the species. The efficiency of the honey bee (*Apis spp.*) as a pollinating agent for red clover has given rise to some controversy, the problem having been confused in most environments by the presence of other pollinating agents in large numbers. However, the consensus of opinion is that honey bees do play a part in increasing the seed set and that under certain conditions they may play a major part in pollination (1, 2, 4, 6, 7, 8, 9, 10). Data concerning seed set in red clover obtained over a four-year period at Moss Vale are presented below. Most of the figures have been obtained incidentally from experiments designed with other objectives, but it is considered desirable to present them forthwith owing to the importance of their implications in regard to the production of red clover seed in this country.

## 2. Material and Methods.

The data were collected in 1937, 1938, 1939, and 1940.

In 1937, about 5,000 spaced plants, representing 20 different strains of various nationalities, were planted at Moss Vale. They were allowed to flower freely, and when the seed was mature three heads from each of 73 plants, selected at random, were harvested, threshed, and the seeds counted. The process was repeated with the same and additional plants in 1938 and 1939.

In 1940, data were obtained from a randomized row experiment with five strains, viz., N.Z. Certified Montgomery Late, W.P.B.S. Pedigree Montgomery Type, Essex Broad Red, Essex Late Flowering, and a composite strain of seven Czechoslovakian Regional types bulked together. The rows were 30 links long, and 2 links apart. Only four of the ten replications were sampled to estimate seed set, 100 heads being taken at random from each row. These were threshed in bulk and weighed, the number of seeds per head for each row being calculated from a weighed sub-sample of 500 seeds. An additional replication was harvested as a whole, in order to obtain a rough idea of the magnitude of the seed yield. Drought conditions prevailed for the first six months after sowing and weeds were very troublesome, in spite of several inter-row cultivations. Heavy rains occurred in December and flowering took place under very good conditions.

## 3. Results and Discussion.

The single plant figures obtained for 1937, 1938, and 1939 are summarized in Table 1. In Table 2 are recorded three lots of comparable figures from other countries. It will be observed that in 1937

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TABLE 1.—THE AVERAGE NUMBER OF SEEDS SET PER HEAD IN RED CLOVER PLANTS AT MOSS VALE IN 1937, 1938, AND 1939.

	Year.					Number of Plants.	Average Number of Seeds per Head.
1937	..	..	..	..	..	73	50·6
1938	..	..	..	..	..	191	10·2
1939—							
Early strains	..	..	..	..	..	60	2·5
Late strains	..	..	..	..	..	400*	16·1

\* Strains bulked.

the seed set per head at Moss Vale was in excess of the highest figures available from overseas sources, whereas the two subsequent years showed poorer results, although the later seed harvest in 1939 was somewhat better.

TABLE 2.—THE AVERAGE NUMBER OF SEEDS SET PER HEAD IN RED CLOVER ELSEWHERE.

Authority.			Country.	Average Number of Seeds per Head.
R. D. Williams (11)	..	..	Wales	47·1
C. O. Jörgensen (4)	..	..	Denmark	26·4
E. Lindhard (5)	..	..	Denmark	36·0
E. Lindhard (5)	..	..	Denmark	7·0

The figures obtained from the 1940 row experiment are set out in Table 3. All five strains have produced a high number of seeds per head. The average yields obtained in several of the recognized red clover seed producing countries are:—Wales 336-450 lb. per acre, Canada 200, Hungary 440, Sweden 251, and U.S.A. 100. At Moss Vale the yield obtained in 1940 was at the rate of 490 lb. per acre. The latter, although a rough estimate, indicates that the yield is of the same order of magnitude as that obtained elsewhere with the aid of humble bees. Wolfe and Kipps (12) state that in Virginia (U.S.A.) it is considered that “when many of the heads have from twenty-five to thirty seeds per head, and the stand is good, the crop is worth harvesting

TABLE 3.—THE AVERAGE NUMBER OF SEEDS PER HEAD IN REPLICATED ROWS OF RED CLOVER AT MOSS VALE IN 1940.

Strain.						Average Number of Seeds per Head.
W.P.B.S., S 123	..	..	..	..	..	61·2
Essex Broad	..	..	..	..	..	60·0
Essex Late	..	..	..	..	..	63·7
N.Z. Cert. Montgomery	..	..	..	..	..	58·2
Czech. Composite	..	..	..	..	..	56·5

for seed." Under such conditions the seed yield is said to range from 1 to 2 bushels per acre. It is clear that under conditions such as existed at Moss Vale in 1937 and 1940 the honey bee is just as effective in pollinating red clover as are the mixed bee populations of other countries.

Efforts to correlate the fluctuating annual results with environmental factors have not been successful. Although the four years concerned have been abnormally dry, the rainfall incidence does not appear to have exerted a determining effect on the seed set. It is considered more probable that the limiting factor has been honey bee activity. This activity may be influenced by:—

- (1) The honey bee population within a radius of a mile or more of the area;
- (2) the quantity and quality of the other honey flora offering;
- (3) the dominant type of activity in the bee hives during the red clover flowering period.

During the 1940 flowering period, three nucleus hives were situated within a few hundred yards of the red clover material at Moss Vale, but in previous years the nearest hive was some distance away.

It is generally accepted that red clover is a pollen rather than a honey plant, although in special circumstances it may fulfil the latter role. By manipulating the three varieties mentioned above, it should be possible to ensure that sufficient visits are paid by bees to the clover crop to give a high setting of seed. Similar methods have proved very successful in increasing red clover seed yield in Czechoslovakia and in certain other countries where the humble bee populations may not be great enough at certain periods to ensure optimal pollination. In Russia, Klingen was able to increase the seed yield of red clover from 178 lb. per acre to 524 lb. per acre over an average of four seasons by placing hives of honey bees alongside selected fields.

Stapel (10) suggests that for Danish conditions from 3 to 5 hives per acre should be provided. Gubin (2) at the Moscow Bee Keeping Research Institute suggests that a minimum of 100 hives should be used as a pollinating nucleus if the seed crops are over one and one-half miles from other bee centres.

The quantity and quality of the other honey flora offering are somewhat beyond the growers' control, but the factor should be borne in mind when siting seed production areas, and care taken that they are not placed close to white clover or similar crops.

It is in the control of the hive itself that the grower has the greatest opportunity of increasing the number of effective bee visits to the crop. It has been shown by Stapel and others that the dominant role of the worker bees may be changed from that of honey collection to that of pollen collection by the following simple measures:—

- (1) Feeding liberally with sugar.
- (2) Increasing the number of brood.
- (3) Maintaining the pollen supply at starvation point by removing quantities from the frames.

It is interesting to observe that the nucleus hives at Moss Vale in 1940 were fed large quantities of sugar throughout the flowering period.

#### 4. Conclusions.

(1) At the Council's Field Station, Moss Vale, N.S.W., the honey bee was found to be a very efficient pollinating agent for red clover, the resulting seed yields being comparable with those obtained in the recognized seed-producing countries.

(2) There was no significant difference in the number of seeds set per head by five representative strains of red clover at Moss Vale in 1940.

(3) It is probable that seed set in the species in Australia could be stabilized at a fairly high level by utilizing hives of honey bees in association with the crop during the flowering period in a manner that has proven successful elsewhere and that is outlined in this report.

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## Studies on the Mitchell Grass Association in South-western Queensland.

### 1. Some Observations on the Response of Mitchell Grass Pastures to Good Summer Rains following the 1940 Drought.

*By R. Roe, B.Sc.Agr.\**

#### *Summary.*

Observations were made on the effect of heavy summer rains on Mitchell grass country after a protracted dry period.

The chief response was from annual grasses, mainly Flinders grass.

The response of mature Mitchell grass was only fair.

There was evidence that the stand of Mitchell grass had been thinned considerably during the dry seasons up to December, 1940.

There was a good germination of Mitchell grass seed, and 1.64 seedlings per square link were recorded.

There was little response from the common herbaceous species. These generally respond to winter rains. There was, however, a fair proportion of annual herbs in the pasture.

Even after exceptionally heavy rains distributed over 5 to 6 weeks, the summer species and soil dried out rapidly.

#### I. Introduction.

These observations were made on a representative area of 600 acres of Mitchell grass plain on the Council's station, "Girruth Plains," Cunnamulla, Q. The area was selected as the site for a grazing trial, and during 1940 was subdivided in preparation for the trial. The area is relatively uniform and the soil is predominantly a heavy brown loam, but towards the southern end and along portion of the western side it merges into a heavy self-mulching greyish clay-loam.

The season 1939-40 was a severe drought and its severity may be seen from the table showing the rainfall at "Girruth Plains" homestead from January, 1939, to May, 1941.

TABLE 1.—RAINFALL IN POINTS AT "GILRUTH PLAINS" HOMESTEAD.

—	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Total.
1939..	104	257	297	172	50	68	72	341	..	96	170	19	1,646
1940..	80	124	11	75	68	9	..	26	40	10	42	201	686
1941..	898	39	488	49	..	..	..	..	..	..	..	..	..

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The total rainfall for the year was 686 points, compared with the annual average for the district of 1,402 points. From August, 1939, to the end of 1940, only in November, 1939, and February, 1940, were registrations of over an inch recorded. These rains produced very little growth on the pasture.

Towards the end of December, 1940, there was virtually no green feed on the plain country. Dry Mitchell grass stubble 6 to 8 inches long and of poor quality was all that was available to the sheep, and they had to be hand-fed. The soil was very dry and cracked. With the advent of good rains, however, a remarkable transformation was brought about. A comparison of Figs. 1 and 2, Plate 1, illustrates the effect of these rains.

## 2. Harvest Data.

In February 1941 a sample cut was taken on the pasture which yielded 17·1 cwt. of hay. Of this yield, by far the greatest proportion was contributed by annual grasses. Samples of the pasture were hand separated into five component groups, and the following table shows the proportion which each contributed to the pasture.

TABLE 2.—BOTANICAL COMPOSITION OF MITCHELL GRASS PASTURE,  
“GILRUTH PLAINS,” FEBRUARY, 1941.

Mitchell Grass.	Other Grasses.	Legumes.	Miscellaneous.	Inert.
%	%	%	%	%
7·9	74·3	0·2	9·7	7·8

The most striking points illustrated by these figures are the preponderance of “other grasses” and the unexpectedly low proportion of Mitchell grass. Each of the components is discussed in some detail below.

### (a) *Mitchell Grass*—

Although the relative yield of Mitchell grass tends to be masked by the high yield of “other grasses” the absolute yield is also low, being only 1·3 cwt. per acre. This low yield is the result of a series of dry summers, culminating in the drought of 1940.

Seedlings of Mitchell grass, although numerous (Table 6), did not contribute much to the yield. Compared with the annuals their development during the six weeks from the beginning of the rains was slow, and in addition they were not as numerous as the annuals. Therefore the yield of Mitchell grass came almost entirely from mature plants. These were not only in poor condition, there being much dead material in almost every tussock, but the number of plants was low. Counts of mature plants were made on both the brown and grey soil types, and in Table 3 these are compared with similar counts taken on two other areas of Mitchell grass in October, 1939.

TABLE 3.—MITCHELL GRASS: PLANT DENSITY AT VARIOUS CENTRES IN OCTOBER, 1939, COMPARED WITH "GILRUTH PLAINS" IN APRIL, 1941.

Station.	"Rockdale," Surat.	"Noondoo," Dirranbandi.	"Gilruth Plains," Cunnamulla.
Annual rainfall—_inches ..	23	18	14
Plants per 25 square links ..	18·8	17·8	Brown loam.
			Greyish clay-loam. 0·6

The marked difference in the Mitchell grass density at "Rockdale" and "Noondoo" compared with "Gilruth Plains" cannot be wholly explained by differences in the mean annual rainfall of the three stations. An examination of Table 4, which shows the rainfall at these three centres during the months November to April inclusive for the three summers preceding the counts, does however show a marked deficiency in the rainfall in the Cunnamulla district. This deficiency, it is suggested, is responsible for the poor condition of the Mitchell grass stand at "Gilruth Plains."

TABLE 4.—RAINFALL IN POINTS AT SELECTED CENTRES FOR THE MONTHS NOVEMBER TO APRIL INCLUSIVE FOR THE FOUR SUMMER SEASONS 1936-40.

Summer (November to April inclusive).	Surat.	Dirranbandi.	Cunnamulla.
1936-37 .. .. ..	1,989	1,519	699
1937-38 .. .. ..	957	518	441
1938-39 .. .. ..	1,566	1,088	934
1939-40 .. .. ..	..	..	479
50-year average for each centre ..	1,394	1,112	841

The summer of 1937-38 was below normal at all three stations, but at both Surat and Dirranbandi two of the three seasons under review were normal to good, whereas the Cunnamulla district was not so well favoured.

The direct effect of the adverse seasons at Cunnamulla was that no young vigorous plants of Mitchell grass (i.e. two- and three-year-old plants) were present, whereas these were plentiful at "Noondoo" and "Rockdale." These young plants, which have a basal diameter of from 4 to 6 inches, were responsible for the high counts at these stations.

Another effect of the drought at "Gilruth Plains" was manifest in the proportion of mature plants that failed to respond to the rains. On two separate areas on the brown loam the mortality was 18 per cent. and 37 per cent. respectively, while on the greyish clay-loam the mortality was as high as 52 per cent. During these observations no attempt was made to differentiate between the three species of Mitchell grass, *Astrebla lappacea*, *A. pectinata*, and *A. elymoides*, all of which are present on the experimental area.

The poor response of those Mitchell grass plants that were not dead may have been due to low summer temperatures. At "Gilruth Plains" for the months of January, February, and March, 1941, the mean maximum temperatures were 9.7°, 4.1°, and 3.7°F. respectively below the normal for those months.

#### (b) Other Grasses—

By far the most common species in this group was Flinders grass, mainly *Iseilema vaginiflorum* Domin. This was present on the brown loam soil type but on the grey soil it was replaced by *Panicum Whitei* J. M. Black, known variously as pepper, pigeon, or sugar grass. Button grass, *Dactyloctenium radulans* Beauv. was associated with the Flinders grass on the brown loam but there was more of it on areas that had been heavily grazed or trampled by stock. Old sheep pads that had become grassed over were almost pure stands of button grass.

Other species that appeared commonly in isolated areas were early spring grass (*Eriochloa* spp.), native millet (*Panicum decompositum* R. Br.), and never-fail (*Eragrostis setifolia* Nees). Occasional species over the whole area were seedlings of Queensland blue grass (*Dichanthium sericeum* (R. Br.) A. Camus), slender chloris (*Chloris divaricata* R. Br.), fairy grasses (*Sporobulus Caroli* Mez and *S. actinocladus* F. Muell.) and *Aristida anthoxanthoides* Henr.

#### (c) Legumes—

The legumes as a group were insignificant. They occurred fairly commonly as small individuals scattered throughout the area but contributed very little to the yield of the pasture.

The chief species were *Rhynchosia minima* (L.) D.C., *Psoralea patens* Lindl. (this occurs very densely in certain areas), *Glycine* spp., *Crotalaria dissitiflora* Benth., and *Indigofera viscosa* L.

#### (d) Miscellaneous—

The herbaceous species, other than legumes, constituted this group. The most common species was cow vine (*Ipomoea lonchophylla* J. M. Black). It was the first species to show on the plain after rain and grew

rapidly. Two other species that occurred commonly were small plantain (*Plantago varia* R. Br.) and *Brachycome melanocarpa* Sond. and F. Muell., but they were small and contributed little towards the yield of the pasture. Other species occurring occasionally were *Sida corrugata* Lindl. var., bladder ketmia (*Hibiscus trionum* L.), wild mulberry (*Malvastrum spicatum* (L.) A. Gray), daisy burr (*Calotis* spp.), *Trichinium incanum* R. Br., pigweed (*Portulaca oleracea* L.), tahvine (*Boerhaavia diffusa* L.), and small *Kochia* and *Bassia* species. There also occurred, although rarely, plants of many other species.

Although the response to the rains was chiefly from the grass species, the contribution by the herbage species of approximately 10 per cent. of the yield must be considered of importance. As a potential source of nutrients some of the herbaceous species must be rated highly. Table 5 gives the chemical composition of field collections of species occurring at "Girruth Plains" or of others closely related thereto.

TABLE 5.—CHEMICAL COMPOSITION OF SOME QUEENSLAND HERBAGE SPECIES COMPILED FROM ANNUAL REPORTS OF THE QUEENSLAND DEPARTMENT OF AGRICULTURE AND STOCK.

(Results as percentages.)

Species.	Crude Protein.	Fat.	Carbo- hydrates.	Fibre.	Ash.	CaO.	P <sub>2</sub> O <sub>5</sub> .
	%	%	%	%	%	%	%
<i>Ipomoea reptans</i> .. ..	22.5	2.5	40.7	20.3	14.0	1.88	0.803
<i>Sida corrugata</i> .. ..	17.9	1.0	42.6	24.3	14.2	3.00	0.550
<i>Boerhaavia diffusa</i> .. ..	18.3	1.1	43.1	19.9	17.6	3.94	0.537
<i>Malvastrum spicatum</i> .. ..	16.9	1.4	33.0	20.1	28.6	3.95	0.768
<i>Portulaca oleracea</i> .. ..	16.2	1.6	31.7	18.1	32.4	2.84	0.399
<i>Salsola Kali</i> .. ..	15.6	1.1	34.1	17.1	32.1	5.21	0.306

The high crude protein, calcium oxide, and phosphorus pentoxide, together with the low fibre in these species, indicates that they are (potentially) rich sources of these nutrients.

#### (e) Inert—

This consisted almost entirely of hard dry blackened stems from old Mitchell grass tussocks.

### 3. Mitchell Grass Seedlings.

Counts of Mitchell grass seedlings were made on six paddocks of the grazing trial. A quadrat one link square was used in counting, but for ease of comparison with the counts of grown plants the results are expressed as seedlings per 25 square links. The number of observations on each paddock varied but was at least 100. Table 6 records the results of these counts.

TABLE 6.—MITCHELL GRASS SEEDLING DENSITY AT "GILRUTH PLAINS,"  
APRIL, 1941.

Paddock.	Seedlings per 25 square links.		
C3 .. ..	32	..	..
C4 .. ..	33	..	..
C7 .. ..	41	..	..
Mean .. ..	..	35	
D3 .. ..	29	..	..
D6 .. ..	22	..	..
Mean .. ..	..	25	
I7 .. ..	..	15	

Paddocks C and D were on the typical brown loam, whereas I7 was on the grey soil type.

This seedling population is regarded as satisfactory. Taking the intermediate figure of 25 seedlings per 25 square links, a 75 per cent. survival would result in a stand comparable with that at "Rockdale," Surat, in 1939. Although a 75 per cent. survival would be high and may not be attained, given a favourable 1941-42 summer the Mitchell grass on "Gilruth Plains" is expected to improve greatly.

#### 4. Length of Growing Period of Pasture.

One noticeable feature of the 1940-41 summer at "Gilruth Plains" was the relatively short growing season after the first rains, and the rapidity with which the pasture dried off. At five to six weeks after the rains began, most of the Mitchell grass was flowering and seeding. At seven weeks, seed heads were beginning to dry and basal leaves were dry and curled. Seed had matured. The drying of the seed heads at this stage was more pronounced in *Astrebla lappacea* and *A. pectinata* than in *A. elymoides*. At eight weeks seed had begun to fall freely. Thus, in two months from the break in the season, during which time 937 points of rain were recorded, the Mitchell grass shot, seeded, and was rapidly drying off.

Other grass species had also dried off. Button grass had seeded and the inflorescences had disintegrated and fallen. Flinders grass dried almost as quickly, and as it matured a heavy infestation with a smut, *Cintractia iseilematis*, became evident. *Panicum Whitei* dried off most rapidly of all but seeded prolifically.

The herbaceous species remained green longer than the grasses, and the small legume *Rhynchosia minima* was still green and in active growth ten weeks after the opening rains, two weeks after the dominant plants were mature and dry.

Despite the exceptionally heavy rains during January, when portion of the plain country was flooded for days, by the end of February the soil was so dry that some cracks up to three inches wide and at least three feet deep had developed over most of the plain.

Conditions were dry by mid-March when an additional fall of almost five inches of rain revived the pasture. There was a fresh growth of mature Mitchell grass, and by mid-April some plants (estimated at 5 per cent.) were seeding a second time. This March rain was of great benefit to the seedlings of Mitchell grass, for they grew considerably, and approximately 10 per cent. flowered.

A fresh crop of Flinders grass also followed the March rains. There was some germination of seed, but most of the new growth was produced from a second growth of the established plants. This second crop was seeding by mid-April, and as it matured *Cintractia* again became apparent. The early spring grass (*Eriochloa* spp.) also made a second growth.

Button grass and *Panicum Whitei* responded little to the March rains, and these rains proved destructive as far as button grass feed was concerned. The dry button grass leaves and seed heads were beaten into the soil and so rendered useless as feed. The dry Flinders grass was not so affected, and this, together with the fact that it holds its seed when dry whereas the button grass does not, indicates its superior value as dry feed, and it is as dry feed that these two species have most value.

### 5. Acknowledgment.

Grateful acknowledgment is made of the help of Mr. G. H. Allen, Field Assistant at "Gilruth Plains," in supplying some of the information contained in this report.

## A Note on the Use of Drainage Water.

By A. L. Tisdall, M.Agr.Sc.\*

### 1. Introduction.

In the irrigation settlements of Mildura, Red Cliffs, Merbein, and Coomealla, the drainage schemes provide each property with facilities for removing underground drainage water. Large subsurface gravitational mains carry the water away from the horticultural settlements, either back to the Murray River, or out to natural depressions in the adjoining country. The mains have been functioning for some years, the water so removed coming from agricultural tile drains on the properties; and in all the above areas extension of this internal drainage is proceeding rapidly.

The position has now arisen that the main drains, particularly during irrigation periods, are carrying large quantities of water away from the irrigation settlements, and the need for an examination of this water is two-fold. Firstly, it is becoming increasingly necessary to conserve water in the Murray Valley, as more and more land is opened up for irrigation. Secondly, these drainage effluents could command, either gravitationally or with a low lift, considerable areas of dry country which at present are undeveloped, and for which water could not readily be obtained in any other way. Much of this land is heavy river flat country, while some of it is Mallee land on a higher contour. These areas could possibly be used for the production of fodders, pastures, and vegetables, provided an adequate supply of water reasonably free from salt was assured.

### 2. Procedure.

In May, 1940, following a request from the Mildura Development Committee, an inquiry into the possible use of this drainage water was commenced. During the period May 1940 to June 1941, a survey was made of the salt content of the principal main drainage outfalls. Some of these were sampled and examined at least weekly for the full period; with others the tests covered the latter part only. The drain outfalls examined, together with the period of record, are shown below:—

- A. *Red Cliffs*.—Complete records were obtained on the main drains known as:—Cardross, Red Cliffs South-East, Red Cliffs Central, Red Cliffs North-West. The Red Cliffs East H Scheme was tested weekly from January to June, 1941.
- B. *Merbein*.—Complete records were obtained at West Merbein, and records from December 1940 to June 1941 at South Merbein.
- C. *Mildura*.—The following outfalls were tested regularly from October to December, 1940, and intermittently from January to March, 1941:—Trymple Valley, Mildura North-West, Mildura North, Mildura East, Koorlong A, Koorlong B, Koorlong C.
- D. *Coomealla* main drain was examined weekly from November 1940 to June 1941.

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\* An officer of the Commonwealth Research Station, Merbein.

The method of testing was to determine the chloride content, and to express the salt content as parts per 100,000 of sodium chloride. No other soluble salts were determined, but as sodium chloride is the dominant one, it may be taken as an indication of the value of the water. The concentrations were then graphed for the whole year. Measurements of quantity of effluent were available for Cardross and Red Cliffs South-East, and were included in the graphs. The corresponding irrigation periods on the land served by each drainage unit were also included. Typical curves illustrating the relation between concentration, effluent, and irrigation period are shown in Fig. 1, while Fig. 2 gives the range of concentration.

### 3. Results and Conclusions.

1. The salt content is at its lowest while the flow of drainage water is greatest, which occurs soon after the start of irrigation. The concentration then rises steadily as the effluent decreases (Fig. 1).
2. Red Cliffs North-West, Central, and Cardross, showed a salt content of less than 40 parts per 100,000 for the whole period, and are suitable for pastures or fodders.
3. Red Cliffs South-East and East H were quite satisfactory except for periods of small flow in July 1940 and March 1941. In any case, these periods of low flow would not be satisfactory for irrigation from a quantity point of view.
4. The Coomealla main was below 40 parts during the period of examination.
5. The West Merbein drain did not rise above 45 parts, and was approximately 20 parts in peak periods.
6. The South Merbein main was always over 40 parts; and also the concentration rose much higher at times, as the flow was intermittent.
7. Of the Mildura mains, all except two were too salty to be considered, ranging generally from 50 to 500 parts (Fig. 2). Mildura East and Koorlong B were the exceptions, the range rarely exceeding 60 parts.
8. It is of considerable interest to note that the concentration in the main drains reflects to a certain extent the types of soil on the area served. Thus at Red Cliffs and Coomealla, which contain the greatest proportion of light soils, the drains show the lowest concentrations. Merbein contains more of the heavier types and the concentrations are higher. Mildura area has a large proportion of heavy soils, and the concentrations shown are much higher still. This may mean that the light soils have much less salt, but there is also the factor that the heavier soils drain much less freely. Less water will reach the drains, and will tend to be more salty. It should also be borne in mind that Mildura, and to a lesser extent, Merbein, are much older settlements. Thus they have had much more time to become salty under irrigation conditions, as in general the drainage schemes are only about five years' old.

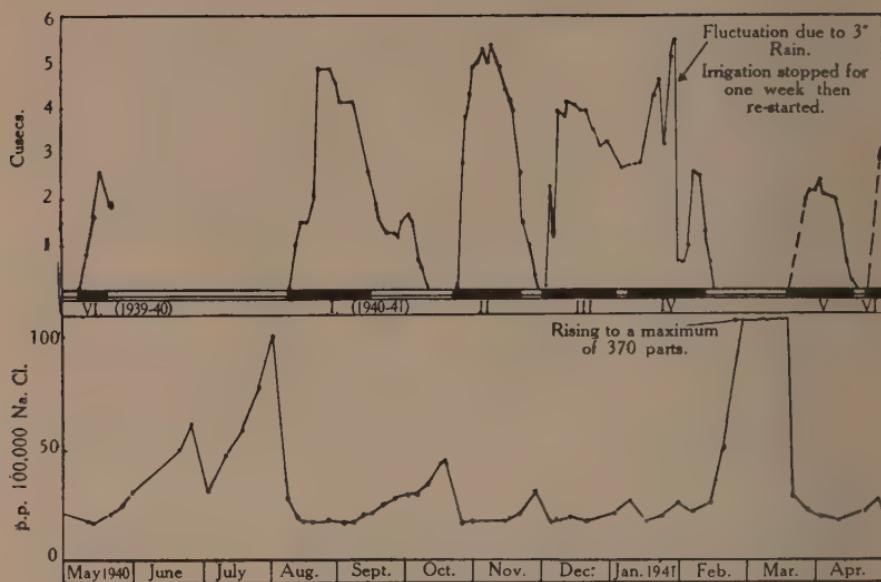


FIG. 1.—Graphs for the Red Cliffs South-East Scheme showing the relation between period of irrigation, and the salt concentration and quantity of the drainage effluent. The lower curve shows salt concentration and the centre band the period of irrigation, each irrigation being numbered. The upper curve shows the quantity of water in cusecs. In many cases the flow is too small to record on the Dethridge meter, but large enough to be sampled.

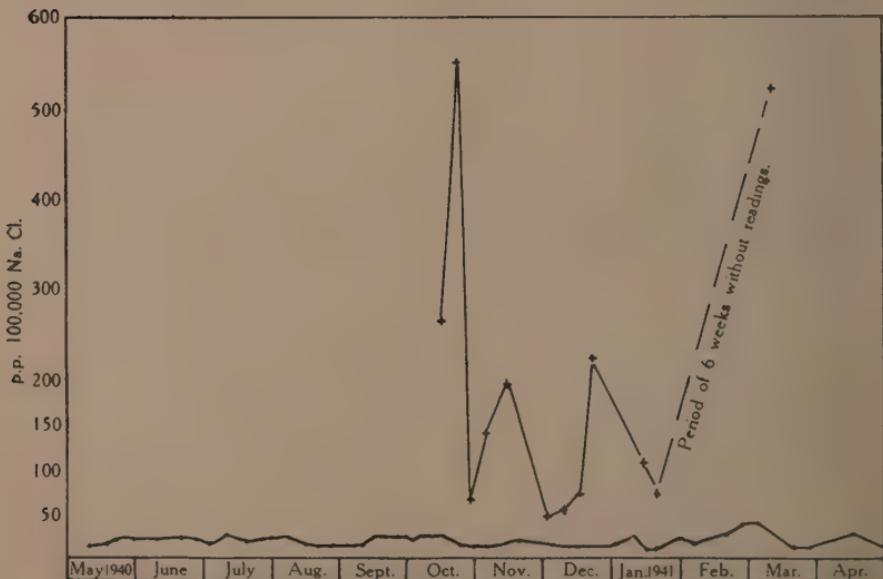


FIG. 2.—Graph showing the fluctuations in salt content in drainage water. The top graph represents the Irymple Valley Scheme, and the lower one represents the Cardross Main. Both show the decrease in concentration due to irrigation, but in the Cardross one the fluctuations are not so marked and do not show plainly with the scale used.

#### 4. Acknowledgments.

The author wishes to thank sincerely the officers of the State Rivers and Water Supply Commission, Red Cliffs, the First Mildura Irrigation Trust, and the Water Conservation and Irrigation Commission, Curlwaa, who were responsible for collection of many of the samples; and Mr. J. P. Sharpe of the Commonwealth Research Station, Merbein, who assisted with the determinations.

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## Resistance of Australian Timbers to Attack by Marine Borers.

By H. B. Wilson, B.Sc. (Hons.), A.A.C.I.\*

Turpentine (*Syncarpia laurifolia*) is frequently specified for marine piling in Australian waters owing to its high resistance to shipworm damage. As supplies of turpentine are limited it should be conserved during the present critical period for the most exacting uses and other timbers substituted where possible. There are fortunately some fairly satisfactory substitutes that can be used in the waters of New South Wales and Queensland, where marine borer damage is so severe, but the use of these timbers or of turpentine may, however, be quite unnecessary in the harbours of Victoria and Tasmania. The damage due to marine borers in these waters is much less than that in New South Wales and Queensland, and timbers such as messmate stringybark (*Eucalyptus obliqua*) which can claim no high resistance to marine borers have given excellent service as piles on the south Victorian and Tasmanian coasts.

For these southern waters, any of the timbers listed in the A and B strength groups in the "Handbook of Structural Timber Design" can be considered suitable for use as piling, although it cannot be claimed that the performances of all of them will be equally good. They will however be good enough in most cases to justify the conservation of turpentine and similar timbers. The exclusive use of these highly resistant timbers for piling is not warranted under the present circumstances.

The lists given below have been compiled therefore to indicate for each State the timbers available in quantity that can be regarded as suitable for piling in marine structures.

In New South Wales and Queensland only a few highly resistant timbers can be used as piling. But in Victoria, Tasmania, and South Australia the timbers listed and any other heavy hardwoods that grow in New South Wales and Queensland should give reasonable service. For this reason the Victorian list contains New South Wales and Queensland timbers which have not been listed for those States.

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\* An officer of the Division of Forest Products.

AUSTRALIAN TIMBERS THAT ARE SUITABLE FOR MARINE PILING.  
*Queensland and New South Wales.*

Species.		Strength Group.	Remarks.
Standard Trade Common Name.	Standard Trade Reference Name.		
Turpentine ..	<i>Syncarpia laurifolia</i> ..	B	Grows in Queensland and New South Wales
Swamp box ..	<i>Tristania suaveolens</i>	B	Less plentiful than the others
Satinay ..	<i>Syncarpia hillii</i> ..	C	Only one source, namely, Fraser Island, Queensland
Brush box ..	<i>Tristania conferta</i> ..	B	Grows in Queensland and New South Wales
Forest redgum ..	<i>Eucalyptus tereticornis</i>	B	Grows in New South Wales and Queensland

*Victoria and Tasmania.*

Messmate stringybark	<i>Eucalyptus obliqua</i> ..	C	Grows in Victoria and Tasmania
River redgum ..	<i>Eucalyptus rostrata</i> ..	B	Grows in Victoria
Yellow stringybark	<i>Eucalyptus muelleriana</i>	B	Grows in Victoria
White stringybark ..	<i>Eucalyptus eugenioides</i>	B	Grows in Victoria
Coast grey box ..	<i>Eucalyptus bosistoana</i>	A	Grows in South-eastern Victoria
Silvertop ash ..	<i>Eucalyptus sieberiana</i>	B	Grows in Victoria
Brown stringybark ..	<i>Eucalyptus capitellata</i>	B	Grows in Victoria
Southern bluegum ..	<i>Eucalyptus globulus</i>	B	Grows in Victoria and Tasmania. Not very plentiful now

*Other Timbers Available from New South Wales.*

Ironbarks ..	<i>Eucalyptus</i> — <i>paniculata</i> .. <i>siderophloia</i> .. <i>crebra</i> ..	A A A	
Grey box ..	<i>Eucalyptus hemiphloia</i>	A	
Tallowwood ..	<i>Eucalyptus microcorys</i>	A	
White mahogany ..	<i>Eucalyptus acmenioides</i>	B	
Grey gum ..	<i>Eucalyptus punctata</i>	A	
Spotted gum ..	<i>Eucalyptus maculata</i>	B	

NOTE.—These timbers are not suitable for use in the heavily-infested waters of New South Wales and Queensland, but will give excellent service in Victoria, Tasmania, and South Australia

*Western Australia.*

Jarrah ..	<i>Eucalyptus marginata</i>	C	Grows in Western Australia
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For northern Western Australian harbours where severe marine borer attack may have been experienced, see list for New South Wales and Queensland.

AUSTRALIAN TIMBERS THAT ARE SUITABLE FOR MARINE PILING—  
*continued.*

*South Australia.*

Species.		Strength Group.	Remarks.
Standard Trade Common Name.	Standard Trade Reference Name.		
Messmate stringybark	Eucalyptus obliqua ..	C	Small local supply. Plentiful in Victoria and Tasmania
Brown stringybark ..	Eucalyptus capitellata	B	Small local supply
River redgum ..	Eucalyptus rostrata ..	B	Small local supply
Jarrah .. ..	Eucalyptus marginata	C	From Western Australia

Other timbers—Any timber included in the Victorian list.

It would be advisable, before specifying a timber for piling for any particular project, to consider carefully the conditions in the locality where the piling will be used. The Division of Forest Products will be glad to assist in the selection of a timber or group of timbers to suit any given locality.

# The Card Sorting Method Applied to the Identification of the Commercial Timbers of the genus *Eucalyptus*.

By H. E. Dadswell, D.Sc., A.A.C.I.\* and Audrey M. Eckersley, M.Sc.\*

## I. Introduction.

Identification keys based on the dichotomous pattern have always been considered unsuitable, because sooner or later it is necessary to make a choice between certain alternatives where such a choice is extremely difficult. If the wrong alternative is taken then, correct identification may not be possible. In 1938 Clarke (3) outlined the use of the multiple-entry perforated-card method in wood identification. By employing such a method the difficulties inherent in the dichotomous key can be readily overcome, because there is no need to use the diagnostic features in any specified order—the most positive features can be employed early—and it is a simple matter to allow for possible variations in any particular feature in any one species. As a result, the application of the card-sorting method to problems associated with wood identification has been greatly extended.

In the work on the identification of Australian timbers, three separate card-sorting schemes, based on the method outlined by Clarke, have been developed. These are applicable to the separation of (i) pored timbers (hardwoods) of Australia and other parts of the world, (ii) certain family groups of pored timbers, and (iii) the non-pored timbers (coniferous woods). Considerable experience with these identification schemes over a period of two years has definitely established their value. They have fulfilled the two prime requirements laid down by Clarke for a desirable key, namely, (a) simplicity in operation and (b) elasticity in allowing the inclusion of additional species without disrupting the main framework. These schemes have been, and are being, slowly extended to cover the commercial timbers of the world.

In Australia, a large number of commercial timbers belong to the one genus the genus *Eucalyptus*. In developing the card-sorting scheme for pored timbers referred to above, the more important eucalypts were included, but as might be expected in such a broad scheme, these eucalypts can be separated only into several distinctive groups. Whereas in general it is unnecessary to go beyond the genus or a group within the genus for most identification work, in Australia at least it is never sufficient to state that a sample submitted for identification is a eucalypt, the question that must be answered is, "What eucalypt?" It is easy to realize the difficulties likely to be encountered in answering such a question when it is considered that there are some 600 species and varieties of the genus *Eucalyptus*, although only about 100 of these are at present of commercial importance.

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It has been our object over the past 10 years to develop some type of key that would prove effective for the identification of the members of this genus. To this end, certain keys covering a number of commercial eucalypts were developed and published (4, 5). In these keys some attempt was made to take account of possible variations in certain features within any one species, and this attempt succeeded to a certain degree when allowance was made for variations in density, colour, vessel size, vessel number, &c. It was impracticable, however, to allow for all the possible variations on the finer details of structure. It has been considered for some time that some revision of these keys was necessary, and it was decided to explore the possibilities of the card-sorting scheme as applied to the identification of the members of one large genus.

It was realized at the outset that in such an application the usual diagnostic anatomical features would be of little value, since they would be constant throughout the genus, and it would be necessary to investigate and employ other features generally considered of little diagnostic value. With the use of such features there was little likelihood of great variation, and great likelihood of much overlapping. Therefore, before any definite scheme could be put forward, it was necessary to make a survey of those physical properties and anatomical features considered most likely to prove of value, not forgetting in such a survey that minor details of structure should not be overlooked. It was further necessary to make a complete examination of all the available specimens of the commercial species to be included, and in order to cover possible variations at least 10 specimens of each species were examined in detail.

On the basis of these investigations, during which the various commercial eucalypts were fully examined in certain fairly well-defined groups, a list of features was drawn up and a special punched sorting card designed and printed (see Fig. 1.). Applying the detailed information obtained, 76 cards duly notched for appropriate physical properties, anatomical features and geographical distribution have been prepared. The eucalypt groups investigated included the ashes, stringybarks, gums, ironbarks, boxes, bloodwoods, and the Western Australian species. The grouping of the eucalypts in this manner has been common practice based in general on bark characteristics, although in a few cases it refers to the timber type.

## 2. List of Features Used Together with Descriptive Notes.

(Compare Fig. 1.)

### (a) Geographical Distribution:

1. Queensland.
2. New South Wales.
3. Victoria.
4. Tasmania.
5. South Australia.
6. Western Australia.
7. Northern Territory and New Guinea.

ALPHABETICAL KEY USING SPEC. NAMES		LOCALITY		TURBIDITY OR DILN. OF ALG. EXTRACT		COLLUM CHANGE ON FUMING		FIBRES		SPECIAL TESTS		COARSENESS		PARENCHYMA		SPARAE		PARATRACHEAL		ALIFORM & OR CONFLUENT		APOTRACHEAL		CRYSTALS IN CHAMBERED CELLS		ABUNDANT		EXTRAN MATT. COMMON																																
► QUEENSLAND	► NEW GUINEA	► NEW SOUTH WALES	► VICTORIA	► TASMANIA	► SOUTH AUSTRALIA	► WEST. AUSTRALIA	► N. TERR.—NEW GUINEA	► B. DENS. < 30 LB./CU. FT.	► B. DENS. 30-40 "	► B. DENS. 40-50 "	► B. DENS. > 50 "	► CUT ON END SURFACE, HORNY AND SHINY	► GUAVERNS PROMINENT	► GR. RINGS WELL-MARKED	► GRAIN REL. STRAIGHT, NOT WAVY OR CURLY	► GREASENESS	► WHITE DEPOSITS IN VESSELS	► ALKALINITY OF ASH HIGH	► FE. CL. TEST +	► WALL (DIA.) OF LUMEN WALL V. THICK LUMEN INCONSPICUOUS	► FAIRLY ABUNDANT	► ABUNDANT	► ALIFORM & OR CONFLUENT	► APOTRACHEAL	► CRYSTALS IN CHAMBERED CELLS	► ABUNDANT	► NO. / SQ. M. (1' x 1')	► 7 CELLS	► 12 CELLS	► 25 CELLS	► 44 CELLS	► 45 CELLS	► 46 CELLS	► 47 CELLS	► 48 CELLS	► 49 CELLS	► 50 CELLS	► 51 CELLS	► 52 CELLS	► 53 CELLS	► 54 CELLS	► 55 CELLS	► 56 CELLS	► 57 CELLS	► 58 CELLS	► 59 CELLS	► 60 CELLS	► 61 CELLS	► 62 CELLS	► 63 CELLS	► 64 CELLS	► 65 CELLS	► 66 CELLS	► 67 CELLS	► 68 CELLS	► 69 CELLS	► 70 CELLS	► 71 CELLS		
► BISERATE PRESENT	► TRISERATE PRESENT	► MORE THAN TRI'S PRESENT	► MAX. HT. OF RAYS— UP TO 15 CELLS	► 16-20 "	► > 20 "	► > 20 "	► TLOSSES FEW	► NO. / 20 SQ. M.M. < 150	► > 250 / 20	► > 350 / 20	► > 300	► MAX. TANG. DIAM. STRONG OBLIQUE TEND.	► IN RADIAL PR's OR MULT's	► IN RADIAL PR's OR MULT's	► GROWTH RINGS: often marked microscopically.	► Parenchyma: forms caps to pores and often lines up in short uniseriate lines.	► VESSELS	► SPECIES <i>E. salmonophloia</i> F.T.W.	► COLOUR	► ASH	► COMMON NAME	► SALMON GUM dark red with a purplish tinge.	► Basic density range = 49.8 - 62.0 lbs./cu. ft.	► Growth rings: often marked microscopically.	► Parenchyma: forms caps to pores and often lines up in short uniseriate lines.	► RAYS	► EXTRAM MATT. COMMON	► ABUNDANT	► NO. / SQ. M. (1' x 1')	► 7 CELLS	► 12 CELLS	► 25 CELLS	► 44 CELLS	► 45 CELLS	► 46 CELLS	► 47 CELLS	► 48 CELLS	► 49 CELLS	► 50 CELLS	► 51 CELLS	► 52 CELLS	► 53 CELLS	► 54 CELLS	► 55 CELLS	► 56 CELLS	► 57 CELLS	► 58 CELLS	► 59 CELLS	► 60 CELLS	► 61 CELLS	► 62 CELLS	► 63 CELLS	► 64 CELLS	► 65 CELLS	► 66 CELLS	► 67 CELLS	► 68 CELLS	► 69 CELLS	► 70 CELLS	► 71 CELLS
► BISERATE PRESENT	► TRISERATE PRESENT	► MORE THAN TRI'S PRESENT	► MAX. HT. OF RAYS— UP TO 15 CELLS	► 16-20 "	► > 20 "	► > 20 "	► TLOSSES FEW	► NO. / 20 SQ. M.M. < 150	► > 250 / 20	► > 350 / 20	► > 300	► MAX. TANG. DIAM. STRONG OBLIQUE TEND.	► IN RADIAL PR's OR MULT's	► IN RADIAL PR's OR MULT's	► GROWTH RINGS: often marked microscopically.	► Parenchyma: forms caps to pores and often lines up in short uniseriate lines.	► VESSELS	► SPECIES <i>E. salmonophloia</i> F.T.W.	► COLOUR	► ASH	► COMMON NAME	► SALMON GUM dark red with a purplish tinge.	► Basic density range = 49.8 - 62.0 lbs./cu. ft.	► Growth rings: often marked microscopically.	► Parenchyma: forms caps to pores and often lines up in short uniseriate lines.	► RAYS	► EXTRAM MATT. COMMON	► ABUNDANT	► NO. / SQ. M. (1' x 1')	► 7 CELLS	► 12 CELLS	► 25 CELLS	► 44 CELLS	► 45 CELLS	► 46 CELLS	► 47 CELLS	► 48 CELLS	► 49 CELLS	► 50 CELLS	► 51 CELLS	► 52 CELLS	► 53 CELLS	► 54 CELLS	► 55 CELLS	► 56 CELLS	► 57 CELLS	► 58 CELLS	► 59 CELLS	► 60 CELLS	► 61 CELLS	► 62 CELLS	► 63 CELLS	► 64 CELLS	► 65 CELLS	► 66 CELLS	► 67 CELLS	► 68 CELLS	► 69 CELLS	► 70 CELLS	► 71 CELLS
► BISERATE PRESENT	► TRISERATE PRESENT	► MORE THAN TRI'S PRESENT	► MAX. HT. OF RAYS— UP TO 15 CELLS	► 16-20 "	► > 20 "	► > 20 "	► TLOSSES FEW	► NO. / 20 SQ. M.M. < 150	► > 250 / 20	► > 350 / 20	► > 300	► MAX. TANG. DIAM. STRONG OBLIQUE TEND.	► IN RADIAL PR's OR MULT's	► IN RADIAL PR's OR MULT's	► GROWTH RINGS: often marked microscopically.	► Parenchyma: forms caps to pores and often lines up in short uniseriate lines.	► VESSELS	► SPECIES <i>E. salmonophloia</i> F.T.W.	► COLOUR	► ASH	► COMMON NAME	► SALMON GUM dark red with a purplish tinge.	► Basic density range = 49.8 - 62.0 lbs./cu. ft.	► Growth rings: often marked microscopically.	► Parenchyma: forms caps to pores and often lines up in short uniseriate lines.	► RAYS	► EXTRAM MATT. COMMON	► ABUNDANT	► NO. / SQ. M. (1' x 1')	► 7 CELLS	► 12 CELLS	► 25 CELLS	► 44 CELLS	► 45 CELLS	► 46 CELLS	► 47 CELLS	► 48 CELLS	► 49 CELLS	► 50 CELLS	► 51 CELLS	► 52 CELLS	► 53 CELLS	► 54 CELLS	► 55 CELLS	► 56 CELLS	► 57 CELLS	► 58 CELLS	► 59 CELLS	► 60 CELLS	► 61 CELLS	► 62 CELLS	► 63 CELLS	► 64 CELLS	► 65 CELLS	► 66 CELLS	► 67 CELLS	► 68 CELLS	► 69 CELLS	► 70 CELLS	► 71 CELLS

FIG. 1.

*Note.*—The knowledge of the origin of a specimen submitted for identification can often be of assistance, although it must be realized that certain eucalypts are widely used in practically every State. For example, the ironbarks, tallowwood, and white mahogany\* are used for poles and piles in Queensland, New South Wales, Victoria, and South Australia, but they are, with the exception of red ironbark, found only in New South Wales and Queensland.

(b) *Physical Properties and Macroscopic Features:*

8. Basic density less than 30 lb./cubic foot (i.e., air-dry density at 12 per cent. M.C. less than 37.5 lb./cubic foot).
9. Basic density between 30–40 lb./cubic foot (i.e., air-dry density at 12 per cent. M.C. between 37.5–50 lb./cubic foot).
10. Basic density between 40–50 lb./cubic foot (i.e., air-dry density at 12 per cent. M.C. between 50–62.5 lb./cubic foot).
11. Basic density above 50 lb./cubic foot (i.e., air-dry density at 12 per cent. M.C. above 62.5 lb./cubic foot).
12. Cut across end grain is hard and horny as with the ironbarks.
13. Gum veins prominent.
14. Growth rings well marked.
15. Grain relatively straight, not wavy or curly.
16. Wood greasy in nature as in tallowwood.
17. White deposits in vessels.
18. Alkalinity of ash high (a chemical test—information not available for every species).

*Note.*—It is suggested that wherever possible basic density (based on the ratio of oven-dry weight to volume soaked) be used. For identification purposes it is most helpful, since the effect of collapse is eliminated. However, for comparison, the equivalent air-dry density ranges have been included (6). Bad collapse in any specimen will raise the air-dry density considerably.

(c) *Colour:*

19. Colour on brown side, that is, white, yellow, straw, brown, etc.
20. Colour on red side, that is pink, pink-brown, red, reddish-brown, etc.
21. Any distinctive colour, as in gimlet.

*Note.*—Allowance has been made for all the colours encountered in this group of timbers, and where there is a large variation in the colour of various specimens of the one timber, the card for that timber has been notched at both 19 and 20. For example, jarrah is a definitely red timber and its card has been notched at perforation 20 only. On the other hand, with a timber like mountain grey gum, which is generally brown or light brown in colour but may be pinkish, perforations 19 and 20 have both been notched. In such cases the exact range of colour to be expected and the usual colour encountered have been indicated on

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\* The common names of the eucalypts referred to in this paper are the standard trade common names adopted by the Standards Association of Australia—Australian Standard O.2—1940.

the card. Certain timbers such as gimlet and powder-bark wandoo, both from Western Australia, have a rather distinctive colour, and so their cards have been notched at perforation 21.

(d) *Burning Splinter Test:*

22. Match-size splinters burn to a full ash.
23. Match-size splinters burn to a partial ash.
24. Match-size splinters burn to a charcoal.

*Note.*—Here again in the preparation of the individual cards due allowance has been made for variation within a species, and in many cases two out of the three possibilities have been notched.

(e) *Vessels:*

25. In solitary arrangement (as in the great majority of the eucalypts).
26. In radial pairs or multiples (as in the bloodwood group).
27. With a strong tendency to oblique alignment but still solitary.
28. With maximum tangential diameter less than  $150\mu$ .
29. With maximum tangential diameter between  $150-250\mu$ .
30. With maximum tangential diameter greater than  $250\mu$ .
31. Numbering less than 150 per area of 20 sq. mm.
32. Numbering between 150 and 300 per area of 20 sq. mm.
33. Numbering more than 300 per area of 20 sq. mm.
34. Tyloses few.
35. Spare.
36. Spars.

*Notes.*—(i) All the eucalypts except those of the bloodwood group (Blakely's *Corymbosae* and *Corymbosae-Peltatae* (1)) have predominantly solitary vessels. The various species of the bloodwoods have a number of vessels in short radial multiples, and therefore the cards for these species have been notched at perforation 26 and not at perforation 25 although solitary vessels do occur.

(ii) Following the procedure of the International Association of Wood Anatomists, tangential diameters of vessels have been measured in all cases, but the mean value has not been used; instead the mean of the ten largest vessels measured has been recorded on the cards and used when notching the perforations for vessel size in the present investigation. From an identification point of view, vessel size is not often of practical significance, since there is a wide variation within the species of this genus. In certain instances, however, it is of value.

(iii) For all the work on the eucalypts, the vessel number has been determined macroscopically over an area of 20 sq. mm. (approximately), using the small circular die of 5 mm. diameter designed for this work by the Queensland Forest Service. The vessel number can be determined quickly and with reasonable accuracy. The number of vessels in a definite area can often be used advantageously in identification, but here again there may be a tremendous range within a species.

(iv) Most eucalypts have abundant tyloses in the vessels of the truewood; only in a few, such as alpine ash and mountain ash, may the tyloses be scarce or absent.

(f) *Rays:*

37. Exclusively uniseriate but including 1- or 2-cell biseriation in 1 per cent. of the total number of rays.
38. Biserial present.
39. Triserial present.
40. More than 3 cells wide (eucalypts seldom have more rays than 3 cells wide but in a few species some 4 to 5 cells wide occur).
41. Maximum height of rays 15 cells or less (See Note below).
42. Maximum height of rays 16-20 cells.
43. Maximum height of rays more than 20 cells.
44. Multiseriate portion of rays less than 7 cells high (see Note below).
45. Multiseriate portion of rays between 7 and 12 cells high.
46. Multiseriate portion of rays more than 12 cells high.
47. Less than 25 per cent. of total multiseriate.
48. Twenty-five per cent. or more multiseriate.
49. Number of rays per sq. mm. less than 75 (determination on tangential surface).
50. Number of rays per sq. mm. more than 75 (determination on tangential surface).
51. Deposits in ray cells abundant, uniformly distributed in cells.
52. Deposits in ray cells not abundant, centrally distributed, i.e., in central cells but absent from marginal cells.
53. Deposits in ray cells sparse and irregular.

*Notes.*—(i) The maximum height of the rays has been determined by counting the cells of the highest rays as revealed by the tangential section and averaging the ten highest. In this connexion, care should be taken not to include those apparently high rays that are obviously two or more rays running together at the margins. Where the rays are more than one cell wide, this running together can be detected because of the appearance of two or more wider portions. At other times, the slight deformity and divergence from normal appearance show clearly that two rays have run together.

(ii) The height of the multiseriate portion of the rays may be determined in the same manner and the average of the ten highest used.

(iii) The number of rays per sq. mm. have not been determined yet, and therefore this feature has not been employed.

(iv) The observation of the deposits in the ray cells is often of definite assistance in the identification of the eucalypts. For example, in silvertop ash the deposits are abundant and uniform in practically every cell, on the other hand, in yellow stringybark they are more concentrated in the central cells of the rays or completely absent. The great majority of the red-coloured timbers have abundant deposits in the ray cells, whereas certain of the pale coloured timbers have few if any cell contents in the rays.

## (g) Special Tests:

- 54. Turbidity on dilution of alcoholic extract.
- 55. Colour change on fuming with ammonia.
- 56. Positive ferric chloride test using aqueous extract.

*Note.*—Allowance has been made for these special chemical tests, but to date they have not been applied to all the species investigated, and therefore their use must be postponed until the information is available.

## (h) Fibres:

- 57. Thickness of fibre wall less than diameter of lumen.
- 58. Fibre wall very thick, lumen inconspicuous.
- 59. Deposits in fibre lumen.

*Note.*—Thickness of fibre wall is in general related to the density of the timber. However, with karri and jarrah, two timbers of very much the same weight, the very thick fibre wall of the former assists in its separation from the latter.

## (j) Parenchyma:

- 60. Sparse.
- 61. Fairly abundant.
- 62. Abundant.
- 63. Paratracheal.
- 64. Vasicentric.
- 65. Aliform and/or confluent.
- 66. Apotracheal (2).
- 67. Apotracheal—diffuse.
- 68. Crystals present in definite chambered cells.
- 69. Crystals present in ordinary parenchyma cells.
- 70. Crystals abundant.
- 71. Extraneous materials in cells common.

*Notes.*—(i) In the investigation of the eucalypts the question of amount and distribution of parenchyma is important and often assists in identification, but unfortunately both paratracheal and apotracheal types may be present in the one species. Therefore the variation observed may be extremely wide.

(ii) Aliform to confluent parenchyma is common only in the blood-wood group of eucalypts. Only rarely are any other eucalypts distinguished by this feature.

(iii) Two types of apotracheal parenchyma have been referred to, namely, apotracheal and apotracheal-diffuse. This has been done deliberately, because in some specimens parenchyma that was not of the paratracheal type was typically diffuse and in other specimens such parenchyma was concentrated between certain vessels (in a tangential direction) and not typically diffuse. The latter type has been considered as apotracheal and the former for distinction as apotracheal-diffuse.

(iv) Crystals were not present in every species investigated. In some species they were found only in some specimens, in other species they were always typical features.

### 3. Alphabetical Key.

In the top left-hand corner of each card there are seven perforations which have been utilized in developing an alphabetical key so that any species card may be sorted out quickly. For this key the specific name has been chosen. If the first letter of the specific name is between *a* and *l* of the alphabet inclusive, then the first perforation has been notched, if on the other hand it is between *m* and *z* of the alphabet inclusive, the perforation has not been notched. The same procedure has been adopted for the following six letters of the specific name. Thus for the specific name of *Eucalyptus salmonophloia*, perforations 2 and 3 of the alphabetical key have been notched, because the 2nd and 3rd letters of this name are between *a* and *l* inclusive, and perforations 1, 4, 5, 6, 7 have not been notched because the 1st, 4th, 5th, 6th, and 7th letters of the name are not between *a* and *l* inclusive.

When sorting for any particular species card, the spelling of the specific name is followed and the cards that drop out or those retained on the needle are used for further sorting, depending on whether the various letters of the name fall into the *a* to *l* class or the *m* to *z* class. By such means any species card can be quickly separated from the remainder.

### 4. Practical Utilization of the Cards.

When preparing each species card, care has been taken to notch perforations corresponding to all the structural details observed in the examination of the species. In doing this, the variation within a species has been covered as fully as possible. Obviously, when dealing with the numerous species of the one genus, the anatomical variations are not marked and the variation within a species will overlap the variation between species. When this happens it would seem that correct identification is practically impossible. To assist in identifications under such circumstances, a red line has been drawn on the card under the notched perforation corresponding to a feature that is infrequent. For example, in the case of a species which has uniserrate to biserrate rays predominantly and in which only occasionally are triseriate rays observed, the perforations for uniserrate rays and biserrate rays must be notched, but in addition, to cover every possibility, the perforation for triseriate rays must also be notched. However, in the latter case a red line under the notched perforation will indicate that the triseriate ray feature is uncommon.

In using the cards certain of them will drop out after sorting for the particular feature observed. Some of these will show the red danger line under the notch corresponding to this feature. This line should indicate at once that in the species represented the particular feature is uncommon. When carrying out an identification and after sorting on the various observed features a number of cards may be left and some elimination of likely species must be made. The presence of two or more red lines under the features used in sorting should be sufficient to show that the species represented are unlikely possibilities. In this manner there can be some narrowing down of the field.

Further eliminations can be accomplished by consideration of the values obtained in density or vessel number determinations. For example, when the unknown is found to have a basic density of, say,

42 lb./cubic feet, those species that have a lower limit of basic density range above this value may be eliminated. For this reason the 95 per cent. probability range for the density of a species (6) has been recorded on its card, together with details of actual colour ranges, vessel number, and vessel size, when such information may prove important.

Although the great value of the card sorting method of identification lies in the fact that features may be used in any order when sorting, it has been found with the various eucalypts that early sorting on colour, result of burning splinter test, and density, quickly removes a number of cards representing species that can be safely neglected. It is therefore considered that such physical properties should be utilized early, and the suggested order for sorting where no outstanding feature is observed is as follows:—Colour, density, burning splinter test, vessel arrangement, vessel number, State of origin if known definitely, parenchyma, crystals, ray size, ray contents, &c.

One other point needs emphasis. Only positive features should be used. It is quite unsafe to assume that the absence of a particular feature in the specimen under examination precludes various possibilities.

### 5. List of Species Included in the Key.

In setting out this list of the eucalypts examined and included in the key, the botanical names as given in Publication O.2-1940 of the Standards Association of Australia have been employed. Following the name of each eucalypt, the numbers corresponding to the perforations notched on the card for that species have been given. Where the notched perforation on the card has a red line its number has been underlined. Thus by reference to these numbers and either to the card (see Fig. 1) or to the list of features given above there is a record of the physical properties and anatomical features of every eucalypt examined, and it would be a very simple matter for any one interested to duplicate the key.

- E. accedens* W. G. Fitzg.—6, 11, 18, 20, 21, 22, 25, 28, 33, 38, 39, 40, 41, 42, 44, 45, 48, 51, 53, 58, 59, 60, 63, 66, 67, 68, 70.
- E. acmenioides* Schau.—1, 2, 10, 11, 19, 24, 25, 28, 29, 32, 33, 38, 41, 42, 44, 47, 48, 53, 58, 59, 60, 61, 63, 66, 71.
- E. albens* Miq.—See *E. hemiphloia*.
- E. amplifolia* Naud.—1, 2, 9, 10, 11, 20, 24, 25, 28, 29, 31, 32, 38, 39, 41, 42, 43, 44, 45, 47, 48, 51, 58, 59, 62, 63, 64, 66, 67, 71.
- E. amygdalina* Labill.—See *E. austaliana*.
- E. astringens* Maid.—6, 10, 11, 19, 20, 22, 25, 28, 32, 33, 34, 37, 38, 41, 42, 44, 47, 48, 51, 52, 58, 59, 60, 63, 67, 68, 69, 70.
- E. austaliana* R. T. Bak. & H. G. Smith.—2, 3, 4, 8, 9, 10, 13, 19, 20, 24, 25, 28, 29, 31, 32, 37, 38, 39, 41, 42, 44, 45, 47, 48, 51, 57, 60, 61, 63, 66.
- E. bancroftii* Maid.—1, 2, 9, 10, 18, 20, 22, 23, 25, 28, 29, 31, 32, 38, 39, 40, 41, 42, 44, 45, 48, 51, 58, 59, 62, 63, 66, 67, 71.

*E. baueriana* Schau.—1, 2, 3, 10, 11, 19, 20, 24, 25, 28, 29, 31, 32, 38, 39, 40, 41, 42, 44, 45, 48, 51, 52, 59, 62, 63, 66, 67, 68.

*E. bicolor* A. Cunn.—1, 2, 3, 5, 10, 11, 18, 20, 22, 23, 25, 28, 32, 38, 39, 41, 42, 43, 44, 45, 48, 51, 52, 58, 60, 61, 62, 63, 66, 67, 68, 70.

*E. bicostata* Maid., Blakely, & Simmonds.—2, 3, 9, 10, 18, 19, 22, 23, 24, 25, 28, 29, 31, 32, 38, 39, 41, 42, 43, 44, 45, 46, 47, 48, 52, 59, 60, 61, 62, 63, 66, 67, 68, 69, 70, 71.

*E. blakelyi* Maid.—1, 2, 10, 20, 24, 25, 28, 32, 38, 39, 42, 44, 45, 47, 48, 51, 52, 59, 62, 63, 66, 67, 71.

*E. bosistoana* F. v. M.—2, 3, 11, 12, 18, 19, 20, 22, 23, 25, 28, 29, 31, 32, 38, 39, 41, 42, 43, 44, 47, 48, 51, 58, 60, 61, 63, 66, 68, 69, 70.

*E. botryoides* Sm.—2, 3, 9, 10, 11, 18, 20, 23, 24, 25, 28, 29, 31, 32, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 51, 58, 60, 61, 62, 63, 66, 67.

*E. calophylla* R. Br.—6, 9, 10, 11, 13, 18, 19, 22, 26, 29, 30, 31, 34, 38, 41, 42, 44, 47, 53, 57, 62, 63, 64, 65, 66, 67, 68, 69, 70.

*E. capitellata* Sm.—2, 3, 5, 9, 10, 11, 19, 24, 25, 28, 29, 31, 32, 37, 38, 41, 44, 47, 51, 52, 59, 60, 63, 66.

*E. carnea* R. T. Bak.—See *E. acmenioides*.

*E. cinerea* F. v. M.—2, 3, 9, 10, 20, 24, 25, 28, 29, 31, 32, 38, 39, 41, 42, 44, 45, 46, 47, 48, 51, 52, 53, 57, 61, 62, 63, 64, 66, 67, 71.

*E. consideniana* Maid.—2, 3, 9, 10, 11, 19, 24, 25, 27, 28, 29, 31, 32, 38, 41, 42, 44, 47, 48, 51, 53, 59, 60, 61, 63, 66.

*E. coolabah* Blakely & Jacobs.—1, 2, 5, 6, 7, 11, 18, 20, 22, 23, 25, 28, 32, 33, 38, 39, 42, 43, 44, 45, 47, 48, 51, 58, 59, 60, 61, 63, 66, 67, 68, 70, 71.

*E. cornuta* Labill.—6, 10, 11, 18, 19, 22, 25, 28, 29, 31, 32, 38, 39, 41, 42, 44, 47, 48, 51, 58, 59, 60, 63, 67, 68, 70.

*E. corymbosa* Sm.—1, 2, 3, 9, 10, 11, 13, 20, 23, 24, 26, 28, 29, 31, 32, 38, 39, 41, 44, 45, 47, 48, 51, 52, 57, 58, 62, 63, 64, 65, 66, 67.

*E. crebra* F. v. M.—1, 2, 11, 12, 18, 20, 23, 24, 25, 28, 32, 33, 38, 39, 41, 42, 44, 47, 48, 51, 58, 59, 60, 61, 63, 66, 67, 68, 70, 71.

*E. dalrympleana* Maid.—2, 3, 8, 9, 14, 15, 18, 20, 24, 25, 28, 29, 31, 34, 38, 39, 40, 42, 43, 44, 45, 46, 48, 52, 53, 57, 60, 61, 62, 63, 64, 65, 66, 67.

*E. dealbata* A. Cunn.—1, 2, 10, 20, 24, 25, 28, 32, 33, 38, 39, 40, 41, 42, 44, 45, 48, 51, 57, 58, 59, 62, 63, 64, 66, 67, 68, 71.

*E. diversicolor* F. v. M.—6, 9, 10, 18, 20, 22, 25, 27, 29, 30, 31, 32, 38, 39, 42, 43, 44, 45, 46, 47, 48, 51, 58, 60, 61, 63, 64, 66, 67, 69, 71.

*E. dives* Schau.—See *E. australiana*.

*E. elaeophora* F. v. M.—2, 3, 10, 11, 14, 19, 20, 23, 24, 25, 28, 31, 32, 38, 39, 41, 42, 44, 45, 48, 51, 52, 62, 63, 66, 67, 68, 69, 70.

*E. eugenoides* Sieb.—1, 2, 3, 9, 10, 19, 24, 25, 28, 29, 32, 38, 41, 42, 44, 47, 51, 52, 59, 60, 61, 63, 66.

*E. eximia* Schau.—2, 9, 10, 11, 13, 19, 20, 22, 23, 26, 28, 29, 31, 38, 39, 41, 42, 44, 45, 48, 53, 59, 62, 63, 64, 65, 66, 67, 69, 70.

*E. fastigata* Deane & Maid.—2, 3, 9, 15, 19, 20, 23, 24, 25, 28, 29, 31, 32, 34, 37, 38, 39, 42, 43, 44, 45, 47, 53, 57, 59, 60, 63.

*E. fergusoni* R. T. Bak.—See *E. paniculata*.

*E. gigantea* Hook.—2, 3, 4, 8, 9, 14, 15, 19, 20, 23, 24, 25, 28, 29, 31, 34, 37, 38, 41, 42, 44, 47, 53, 57, 60, 63.

*E. globulus* Labill.—3, 4, 9, 10, 14, 16, 18, 19, 22, 23, 25, 28, 29, 31, 32, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48, 52, 53, 59, 60, 61, 62, 63, 66, 67, 68, 69, 70.

*E. gomphocephala* DC.—6, 10, 11, 12, 18, 19, 22, 25, 27, 28, 29, 31, 32, 38, 39, 40, 41, 42, 43, 44, 45, 48, 51, 52, 53, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 70.

*E. goniocalyx* F. v. M.—2, 3, 5, 9, 10, 14, 18, 19, 20, 22, 23, 24, 25, 28, 29, 31, 32, 34, 38, 39, 42, 43, 44, 45, 47, 48, 52, 53, 61, 62, 63, 64, 65, 66, 67, 69.

*E. grandis* (W. Hill) Maid.—1, 2, 8, 9, 10, 20, 23, 24, 25, 29, 30, 31, 32, 37, 38, 42, 43, 44, 47, 48, 51, 53, 57, 60, 61, 63, 66, 67.

*E. haemastoma* Sm.—2, 3, 9, 10, 20, 24, 25, 28, 31, 32, 34, 38, 39, 40, 42, 44, 45, 48, 51, 57, 60, 61, 63, 67.

*E. haematoxylon* Maid.—6, 10, 13, 20, 22, 26, 28, 29, 32, 38, 41, 44, 47, 48, 51, 59, 62, 63, 64, 65, 66, 67, 69, 71.

*E. hemiphloia* F. v. M.—1, 2, 3, 5, 11, 18, 19, 20, 22, 23, 25, 28, 32, 33, 38, 39, 41, 42, 43, 44, 45, 47, 48, 51, 58, 60, 61, 63, 66, 67, 68, 70.

*E. jacksoni* Maid.—6, 9, 20, 24, 25, 27, 29, 31, 38, 39, 41, 42, 43, 44, 45, 47, 48, 51, 52, 53, 57, 60, 61, 62, 63, 66, 67.

*E. leucoxylon* F. v. M.—2, 3, 5, 10, 11, 18, 19, 20, 24, 25, 28, 32, 38, 39, 40, 41, 42, 44, 45, 48, 52, 53, 58, 59, 61, 62, 63, 66, 67, 68, 70.

*E. longifolia* Link & Otto.—2, 3, 10, 11, 18, 20, 23, 24, 25, 28, 29, 31, 32, 38, 39, 41, 42, 43, 44, 45, 46, 48, 51, 58, 59, 62, 63, 64, 66, 67, 71.

*E. macrorrhyncha* F. v. M.—2, 3, 5, 9, 10, 19, 20, 24, 25, 28, 29, 32, 33, 38, 39, 41, 44, 47, 48, 51, 52, 59, 60, 63, 66.

*E. maculata* Hook.—1, 2, 3, 10, 11, 12, 16, 19, 22, 26, 28, 29, 31, 32, 38, 39, 41, 42, 43, 44, 45, 46, 47, 48, 53, 58, 59, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71.

*E. maculosa* R. T. Bak.—2, 3, 9, 10, 18, 19, 20, 24, 25, 28, 29, 31, 32, 38, 39, 40, 41, 42, 44, 45, 48, 51, 52, 57, 59, 60, 61, 62, 63, 66, 67.

*E. maideni* F. v. M.—2, 3, 10, 11, 14, 18, 19, 22, 23, 25, 28, 29, 31, 38, 39, 41, 42, 44, 45, 47, 48, 52, 59, 61, 62, 63, 66, 67, 68, 69, 70, 71.

*E. marginata* Sm.—6, 9, 10, 20, 24, 25, 27, 29, 30, 31, 32, 38, 39, 40, 41, 44, 47, 48, 51, 52, 57, 59, 60, 61, 62, 63, 64, 66, 67, 71.

*E. megacarpa* F. v. M.—6, 9, 10, 18, 19, 22, 25, 27, 28, 32, 38, 41, 42, 43, 44, 47, 51, 52, 53, 57, 59, 60, 63, 67, 68.

*E. melliodora* A. Cunn.—1, 2, 3, 10, 11, 18, 19, 20, 23, 24, 25, 28, 31, 32, 38, 39, 40, 42, 43, 44, 45, 48, 51, 52, 53, 58, 59, 61, 62, 63, 66, 67, 68, 70.

*E. micrantha* DC.—1, 2, 9, 13, 20, 24, 25, 28, 29, 31, 32, 38, 39, 40, 41, 42, 44, 45, 48, 51, 57, 59, 60, 63, 67.

*E. microcorys* F. v. M.—1, 2, 10, 11, 16, 19, 24, 25, 27, 28, 29, 31, 32, 37, 38, 41, 42, 44, 45, 48, 51, 53, 58, 59, 60, 61, 63, 66, 67, 68, 69, 70, 71.

*E. muelleriana* Howitt.—2, 3, 9, 10, 19, 24, 25, 27, 28, 29, 32, 37, 38, 39, 41, 44, 45, 47, 48, 52, 53, 59, 60, 61, 63, 66.

*E. nitens* Maid.—2, 3, 8, 9, 14, 15, 19, 23, 24, 25, 28, 29, 31, 34, 37, 38, 42, 43, 44, 45, 47, 48, 53, 57, 60, 63, 66.

*E. nowraensis* Maid.—2, 10, 20, 23, 26, 29, 31, 37, 38, 42, 44, 47, 53, 62, 63, 64, 65, 66, 67, 68.

*E. obliqua* L'Herit.—2, 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 24, 25, 27, 28, 29, 31, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48, 53, 59, 60, 61, 63, 66, 67.

*E. paniculata* Sm.—1, 2, 11, 12, 18, 19, 20, 21, 22, 25, 28, 29, 32, 33, 37, 38, 41, 42, 44, 47, 48, 51, 58, 59, 60, 61, 63, 66, 68, 70, 71.

*E. parramattensis* Hall.—2, 10, 20, 23, 24, 25, 28, 29, 31, 38, 39, 40, 41, 44, 45, 48, 51, 57, 58, 59, 62, 63, 66, 67, 71.

*E. patens* Benth.—6, 9, 10, 19, 24, 25, 28, 29, 31, 38, 39, 41, 42, 44, 45, 48, 51, 52, 53, 57, 59, 60, 61, 62, 63, 66, 67.

*E. pauciflora* Sieb.—2, 3, 4, 9, 10, 13, 14, 19, 23, 24, 25, 28, 31, 32, 38, 39, 40, 41, 42, 44, 45, 47, 48, 51, 53, 57, 60, 63.

*E. pilularis* Sm.—1, 2, 9, 10, 11, 13, 19, 24, 25, 27, 28, 29, 31, 32, 37, 38, 41, 42, 44, 47, 48, 51, 53, 59, 60, 61, 63, 66, 67.

*E. polyanthemos* Schau.—2, 3, 10, 11, 20, 24, 25, 28, 29, 32, 33, 38, 39, 41, 42, 44, 45, 48, 51, 58, 59, 61, 62, 63, 64, 66, 67, 68, 70, 71.

*E. propinqua* Deane & Maid., *E. punctata* DC.—1, 2, 10, 11, 12, 20, 24, 25, 28, 29, 32, 33, 38, 39, 41, 42, 44, 45, 48, 51, 58, 59, 60, 61, 62, 63, 66, 67, 71.

*E. redundca* Schau. var. *elata* Benth.—6, 11, 18, 19, 20, 22, 25, 28, 32, 33, 38, 39, 40, 41, 44, 45, 48, 53, 58, 59, 60, 63, 66, 68.

*E. regnans* F. v. M.—3, 4, 8, 9, 14, 15, 19, 20, 23, 24, 25, 27, 28, 29, 30, 31, 32, 34, 37, 38, 41, 42, 43, 44, 45, 47, 48, 53, 57, 60, 63, 66.

*E. resinifera* Sm.—1, 2, 10, 11, 20, 24, 25, 27, 29, 31, 32, 38, 39, 41, 42, 44, 47, 48, 51, 52, 58, 59, 62, 63, 64, 65, 66, 67, 71.

*E. robusta* Sm.—1, 2, 9, 10, 20, 23, 24, 25, 28, 29, 31, 32, 38, 39, 40, 42, 43, 44, 45, 46, 48, 51, 59, 62, 63, 64, 65, 66, 67, 71.

*E. rostrata* Schlecht.—1, 2, 3, 5, 6, 7, 9, 10, 20, 24, 25, 28, 29, 32, 38, 39, 40, 41, 42, 43, 44, 45, 48, 51, 52, 57, 58, 59, 61, 62, 63, 64, 65, 66, 67, 71.

*E. rubida* Deane & Maid.—1, 2, 3, 4, 5, 8, 9, 10, 14, 18, 20, 23, 24, 25, 28, 31, 32, 34, 38, 39, 41, 42, 43, 44, 45, 48, 51, 52, 57, 60, 61, 62, 63, 66, 67, 68, 69, 70.

*E. saligna* Sm.—2, 9, 10, 20, 23, 24, 25, 27, 28, 29, 30, 31, 32, 38, 39, 40, 42, 43, 44, 45, 46, 48, 51, 52, 57, 58, 59, 61, 62, 63, 64, 65, 66, 67.

*E. salmonophloia* F. v. M.—6, 11, 18, 20, 21, 22, 25, 28, 33, 38, 39, 41, 42, 44, 47, 48, 51, 58, 59, 60, 61, 62, 63, 66, 67, 71.

*E. salubris* F. v. M.—6, 11, 17, 19, 21, 22, 25, 28, 33, 38, 41, 42, 44, 47, 48, 51, 58, 59, 60, 63, 67, 68, 71.

*E. seeana* Maid.—1, 2, 10, 11, 18, 20, 22, 23, 25, 28, 29, 32, 38, 39, 40, 41, 42, 44, 45, 48, 51, 58, 59, 62, 63, 66, 67, 69, 71.

*E. siderophloia* Benth.—1, 2, 11, 12, 20, 23, 24, 25, 28, 29, 32, 33, 38, 41, 42, 44, 47, 48, 51, 58, 59, 60, 61, 63, 64, 65, 66, 68, 71.

*E. sideroxylon* A. Cunn.—1, 2, 3, 11, 12, 20, 24, 25, 28, 29, 32, 33, 38, 39, 40, 41, 42, 44, 45, 48, 51, 58, 59, 60, 61, 62, 63, 66, 67, 68, 71.

*E. sieberiana* F. v. M.—2, 3, 4, 9, 10, 13, 17, 19, 23, 24, 25, 27, 28, 29, 31, 32, 37, 38, 41, 42, 44, 47, 48, 51, 52, 57, 59, 60, 61, 63, 66.

*E. stuartiana* F. v. M.—2, 3, 10, 18, 19, 20, 24, 25, 28, 29, 31, 32, 38, 39, 40, 41, 42, 44, 45, 46, 48, 52, 53, 59, 61, 62, 63, 66, 67, 68, 69, 70, 71.

*E. tereticornis* Sm.—1, 2, 3, 5, 10, 11, 20, 24, 25, 28, 29, 32, 37, 38, 39, 41, 42, 44, 45, 47, 48, 51, 52, 58, 59, 61, 62, 63, 64, 66, 67, 71.

*E. tessellaris* F. v. M.—1, 2, 6, 7, 10, 11, 13, 18, 19, 21, 22, 26, 28, 29, 32, 38, 39, 41, 42, 43, 44, 45, 46, 48, 51, 59, 62, 63, 64, 65, 68, 70, 71.

*E. trachyphloia* F. v. M.—1, 2, 10, 11, 19, 24, 26, 28, 29, 32, 38, 41, 44, 47, 51, 59, 61, 63, 64, 65, 66, 68.

*E. umbra* R. T. Bak.—See *E. acmenioides*.

*E. viminalis* Labill.—2, 3, 4, 5, 9, 10, 14, 15, 19, 20, 23, 24, 25, 28, 29, 31, 34, 38, 39, 41, 42, 43, 44, 45, 46, 48, 51, 52, 53, 59, 60, 61, 62, 63, 64, 65, 66, 67.

## 6. Classification of Species According to Features.

The above list indicates very clearly the wide variation in the anatomical features of the various eucalypts. As mentioned earlier it would seem at first glance that identification is practically impossible. However, there are a number of small divergences from one species to the next, and when these are added together it is surprising what degree of separation is possible. Obviously by sorting on a particular feature one is able to list all the species with that feature. Thus by sorting at perforation 22 all the species that give a full ash in the burning splinter test may be segregated, or by sorting at perforation 26 all the species that have vessels in radial multiples may be separated from the remainder. Carrying this operation a stage further and using a number of characteristics it was observed that the various species could be separated into groups and with certain combinations of features the groupings obtained corresponded in general to the botanical groupings of Blakely. This will be indicated in the examples given below:—

- (a) All the species examined belonging to Blakely's Corymbosae and Corymbosae-Peltatae were found to have the following characteristics in common, (i) vessels in short radial multiples and (ii) parenchyma commonly aliform to confluent. These species are generally referred to as the "bloodwoods" and the group the "bloodwood group."
- (b) Representatives of Blakely's Series Buxales, Melliodorae, and Heterophloia that were included in the investigation were found to have the following features in common, (i) high density—basic density above 50 lb./cubic foot, (ii) solitary vessels, (iii) abundant crystals in chambered cells, (iv) abundant and uniform deposit in the rays, (v) triseriate rays. These features may therefore be taken as typical of the "box group" of timbers, which general classification covers the 3 Series of Blakely referred to; they are also typical of certain of the "ironbark" timbers but the latter may be distinguished because of their hard and horny cut on end surface.

- (c) The commercial timbers known as the ironbarks fall into Blakely's Series Siderophloiae and Terminales. These timbers can be classified by (i) high density—basic density above 50 lb./cubic foot, (ii) solitary vessels, (iii) abundant and uniform deposits in rays, (iv) colour of timber mostly on red side, (v) cut on end surface hard, horny, and shiny.
- (d) Those commercial timbers classified as gums and falling into Blakely's Series Globulares and Viminales are characterized by (i) abundant parenchyma and (ii) fairly well-marked growth rings. It should be noted here that *E. stuartiana* and *E. elaeophora*, two species of the Series Globulares, are commonly termed boxes but have the anatomical features of the other members of the Series.
- (e) The stringybarks, which are grouped in Blakely's Series Pseudo-stringybarks, white mahoganies, and Pachyphloiae (stringybarks), have the following features in common (i) light-brown to brown in colour (one exception), (ii) low rays—maximum height 15 cells or less, (iii) less than 25 per cent. multiseriate rays, (iv) sparse parenchyma, (v) no crystals, (vi) no definite evidence of growth rings.

Naturally in each group there is a certain amount of divergence from the features listed, but in general all the timbers of the group possess the characteristic features for that group.

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# A Quantitative Investigation of the Steeping Method for the Preservation of Timber. A Modern Application of an Old Process to Australian Mine Timbers.

By H. B. Wilson, B.Sc. (Hons.), A.A.C.I.,\* and J. N. Gregory.\*

## 1. Introduction.

A recent inquiry on behalf of a mining company for a method of preserving round green mine timbers prompted a brief survey of the literature in regard to steeping. This process, in which green or seasoned timber is simply immersed in the preservative liquid for some period of time, is very old indeed. Curiously enough, the available data in regard to the penetrations and absorptions of chemicals in timber treated by this process or any of its modifications are very fragmentary, and are usually presented in a form that renders them difficult of interpretation for conditions other than those which held good when they were obtained. It is, for example, difficult to find any exact figures on the concentration of chemicals in steeped timber. This information is obviously essential for the determination of a suitable treatment schedule.

Steeping in some cheap chemical such as zinc chloride appeared to be the only possible method for cheaply and efficiently treating round, green, mine timber. It was thus decided to undertake a few tests to determine the effects of solution concentrations and times of immersion upon the absorptions and penetrations of preservatives in timber treated by this method.

The steeping process on green timber depends on the diffusion of solute in the treatment solution into the water in the cells of the green timber. Previous work on hot diffusion treatments, where shorter treatment times were used, has shown the possibility of obtaining penetration of preservatives by diffusion in those cases where treatment by pressure or open tank methods do not give satisfactory results with dry timber. It would seem that in cases where such a condition exists, the dry timber is resistant to movement of liquid in a body through it, whereas the ions or molecules of solute in the diffusion treatment are able to penetrate through the green timber quite readily. It is a characteristic disadvantage of a diffusion treatment, however, that the concentration of solute decreases rapidly with distance from the surface of the timber. This, however, is not a serious disadvantage, since the surface of timber requires the most protection, but, for satisfactory results, treating solutions of fairly high concentration have to be used. In this steeping process, with low maintenance and no heating costs, long treatment times can be used. Accordingly, satisfactory treatments seemed probable even with cold solutions.

In regard to other processes and other preservatives, it may be pointed out that oil preservatives are not regarded with favour for underground work because of fears of an increased fire hazard, and that

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treatment processes involving the impregnation of seasoned timber are too costly and in many cases stocks of seasoned timber are not maintained. Furthermore, these treatments do not have the advantage of the simplicity of operation of the steeping process, all requiring at least some technical control which is a disadvantage when mining timbers are treated in the bush.

## 2. Experimental Procedure.

The work was carried out upon specimens of sapwood cut from a green log of messmate stringybark (*Eucalyptus obliqua*) about 12 inches in diameter. The log, with bark intact, was forwarded to the Division on the day that the tree was felled. The log was sawn into discs about 6 inches thick, and, from these discs, billets were sawn off about 6 inches wide, so that each specimen carried a piece of sapwood about 6 inches square by 1 inch thick. The sapwood on most eucalypts is about 1 inch thick.

The sides, truewood (heartwood) face, and ends of each billet, i.e., all faces of the billet except the back, were immediately painted with three coats of red lacquer to prevent drying and also to prevent diffusion through these faces in the subsequent treatment. The bark was not removed until the specimens were about to be immersed. In order to prevent drying of the timber, which retards the rate of diffusion, the preparation of the specimens was completed as rapidly as possible.

The chemical preservatives selected for this work were zinc chloride (fused) and copper sulphate crystals. Both chemicals exceeded 99 per cent. in purity. Aqueous solutions were made in three concentrations for each salt, viz.: 5 per cent., 10 per cent., and 20 per cent. by weight based on anhydrous salt. The solutions were made in enamel buckets and glass jars and were kept at room temperature. The bark was removed from the timber specimens immediately before immersion, and they were then placed in the solutions in which, owing to their greater density, they remained submerged. Two small nails driven into one face of each specimen kept them from coming into contact with each other. The solutions were stirred from time to time to maintain uniform concentrations.

One specimen was treated by each of the following schedules:—

<i>Time.</i>	<i>Solution Concentration.</i>
1 month	.. 5, 10, 20 per cent.
2 weeks	.. 5, 10, 20 per cent.
1 week	.. 5, 10, 20 per cent.

It was not possible to extend the test further owing to the limited time available for the work.

A notable feature of the copper sulphate treatments was the growth of mould on the surface of the 5 per cent. solution. It formed large sheets. It also appeared, though less vigorously, on the 10 per cent. solution, and after about three weeks small colonies developed on the 20 per cent. solution, but their growth was very small indeed. No appreciable effects could be observed in the higher concentrations, but the 5 per cent. solution turned green. It would have been preferable to have carried out this part of the investigation with fresh solutions under aseptic conditions, but, with the limited time available, this was

not possible. The results from the analyses of the copper sulphate treated wood did not differ significantly from those obtained with wood treated with zinc chloride, in spite of the growth of mould on the 5 per cent. copper sulphate solutions.

At the end of the respective treatment periods, the required specimens were removed and allowed to dry in the air.

### 3. Analysis of Treated Specimens.

Each treated specimen was scraped clean of crystals of the treating salt and the sides and ends cut away, leaving a piece about 3 inches square with the full depth of the sapwood. It was felt that this precaution was advisable in order to obtain only wood that had been penetrated from the outer sapwood face. By means of a thin band saw, this central piece of wood was then cut into bands parallel to the growth rings. It was observed that the penetration in the specimen immersed in 20 per cent. copper sulphate solution for one month was very uniform, judging by the colour, but, in the specimens treated for shorter periods, there was a distinct band of heavily impregnated wood about  $1/16$  inch to  $\frac{1}{8}$  inch in thickness on the outside, overlying wood in which the impregnation was much lighter. The layer of sapwood on the specimen immersed in 20 per cent. copper sulphate for one month was cut into four bands of approximately equal width. The layer of sapwood on all the other specimens was cut into five bands of which the first contained only the heavily impregnated outer layer, and the remainder were of equal thickness.

It is of interest to note that this heavily impregnated outer layer was observed, after careful examination, to be limited by the outermost layer of latewood beyond which this layer extended very little if at all. The difference between the concentrations of chemicals in these outer layers and in the inner wood was most marked, as an inspection of the analytical results will show.

The methods of analysis employed were selected mainly because they are rapid and convenient and yet sufficiently accurate for the type of work under discussion. The details of the methods are set out fully in the following paragraphs.

### 4. Methods of Analysis of the Timber for Copper Sulphate and Zinc Chloride.

(a) *Copper sulphate*.—A colorimetric method utilizing the intense blue cuprammonium ion was used for the determination of copper sulphate in the timber. The procedure followed is given below in detail.

The samples are cut up to approximately 10 to 20 mesh, mixed well, and dried in the oven at  $105^{\circ}\text{C}$ . Representative samples of about 1 gramme are weighed out accurately in duplicate into porcelain crucibles. The sample is ignited gently until flaming ceases and then strongly ignited over a Meker burner until glowing ceases and only copper oxide remains. The copper oxide is taken up in a few drops of conc. hydrochloric acid, warming if necessary. The solution is washed into a small beaker, and excess conc. ammonia added. The solution is then made up to a definite volume, according to its strength, judged approximately by the intensity of the cuprammonium blue colour. After filtration, the unknown solution is compared with a standard solution in a Klett or other suitable comparator.

The standards are prepared by making up solutions containing 5, 10, 15, 20, and 25 mg. of copper, to which 1 ml. of 2N hydrochloric acid and excess ammonia are added, and then each made up to 100 ml. The unknown solution is diluted accurately until it falls to a concentration within the range of the standards (judged roughly by eye); the strength of the solution is then determined by colorimetric comparison with the nearest standard. More accurate results are obtained if the higher standards are used for the comparison. This method was tried out with known quantities of copper sulphate in samples of the same timber, and the recoveries of copper sulphate were well within the experimental error of the method, which was as high as 10 per cent, in a few samples, according to the amount of copper in the timber. An analytical accuracy of this order is considered quite satisfactory for the purposes under discussion. The method also had the advantage of being extremely rapid, a single determination taking only about 30 minutes.

(b) *Zinc chloride*.—The determination of zinc in timber is an exceedingly long and tedious procedure, and a simple rapid method could not be found, as in the copper determination. This is mainly because the wood material must be removed by a wet ignition, as zinc and zinc chloride are appreciably volatile at the temperature of a dry ignition. Accordingly, the chloride content of the timber was determined by a modification of a method described by Greaves\*. There is a slight possibility that the  $Zn^{++}$  and  $Cl^-$  might not be present in the timber in exactly equivalent proportions because of hydrolysis and evolution of HCl during drying, but, as the hydrolysis is very small, it is doubtful whether the acid concentration would reach a sufficiently high value for evolution of HCl, if the samples are only air-dried.

The method used for determination of chloride is as follows: The air-dry sample is cut up to about 10 to 20 mesh, well mixed, and duplicate samples of from one to four grammes taken, according to the amount of zinc chloride in the timber. A separate sample is weighed into a weighing bottle for drying in the oven to determine the moisture content, since all results are expressed as percentages on the oven-dry weight of the timber.

The samples for analysis are transferred to platinum dishes, and between 5 and 12 ml. of 15 per cent, caustic soda are added according to the size of the sample. The liquid is then evaporated off in a water bath or Argand burner, and the dish held over a small burner flame until its contained material fuses and then chars. At this stage, one gramme of potassium nitrate per gramme of sample is added slowly whilst still heating and stirring to obtain uniform ignition. The char is rapidly oxidised by the nitrate and leaves a yellow paste which solidifies and becomes white on cooling. The residue is dissolved by warming with distilled water, and transferred with washing, first with water and then with 2N nitric acid, to a tall 400-ml. beaker; further nitric acid is added until the zinc oxide is all dissolved and the solution is acid. The solution is boiled for two or three minutes, and excess calcium carbonate added to make the solution neutral. After cooling, the chloride is titrated with N/10 silver nitrate, using potassium chromate indicator. The end-point is slightly less sensitive than the usual end-point of this

\* Greaves, C. (1933).—Leaching tests on water-soluble wood preservatives. Dept. of Interior, Canada. Forest Service Circ. No. 36.

reaction, and no reason could be discovered for the abnormality. The decrease in accuracy, however, is negligible. Fluorescein indicator was tried, but under these circumstances was less sensitive than the chromate.

The method was tried out with known quantities of zinc chloride in the same timber, and recoveries suitable for the particular requirements (103 to 104 per cent.) were obtained. The method is more accurate than the copper determination, and it can be carried out with almost equal rapidity.

The results of the analyses are given in Tables 1 and 2. In these tables the average result is given for the two duplicate analyses carried out with each sample band. The results are so arranged that the absorptions from the outside face of the sapwood inwards are obtained by reading the table horizontally from left to right.

A photograph of zinc chloride treated specimens is shown (see Plate 2). These specimens were oven-dried, when the well-known darkening of the *heavily* impregnated wood took place. The photograph clearly shows the uniform penetration in the specimen immersed for one month in 20 per cent. zinc chloride solution, and also the outer band of heavily impregnated wood which was present on the specimens treated for shorter periods. The results obtained with copper sulphate were almost identical and did not differ in any essential features.

### 5. General Description of Results.

The penetration of the salts showed a fair degree of uniformity inside the heavily impregnated band, in those specimens immersed for one month in either chemical. It was observed, however, that cross sections (end grain section) of those specimens that were immersed for shorter periods were blotchy in appearance owing to adjoining areas and "islands" of varying degrees of impregnation. The reason for this blotchiness is not clear.

It can also be seen from the tables that the penetrations and absorptions do not appear to be proportional to the time of treatment. In the outer layers, there is scarcely any difference between the one and two weeks treatment with both salts, whilst the four weeks treatments are much above the others, especially with regard to the concentrations in the inner layers. This is also shown by visual observation of the penetrations (see Plate 2). The concentration in the outer layers for both salts is in every case quite large, and it appears that the extra time is required to carry this concentration to the interior of the timber, but it is not known why the absorptions in the one and two weeks treatments should be so similar. It also seems that zinc chloride is absorbed more readily than copper sulphate. Comparison of the second and third layers will show this; it seems that more zinc chloride enters the timber, but, with regard to actual depth of penetration, zinc chloride is only a little better than copper sulphate.

In every sample except 5 per cent.  $\text{CuSO}_4$  for two weeks, there was considerable penetration of salt right to the furthest layers of sapwood adjacent to the truewood.

## 6. Absorptions and Penetrations in Relation to Actual Service.

From a practical point of view, the amounts of chemicals absorbed and the penetrations obtained are very satisfactory. In the specimens treated in 5 per cent. solutions for one week, the concentrations of dry chemical at a depth of half an inch from the surface are approximately 0.4 per cent. This is equivalent to 0.1 to 0.2 lb. of dry salt per cubic foot of treated wood. The concentration increases rapidly towards the outer surface.

Zinc chloride has been successfully used for the preservation of underground and aerial structural wood work with absorptions of  $\frac{1}{2}$  lb. to 1 lb. per cubic foot. The service life of such treated wood is certainly much in excess of five years where leaching is not severe. For timber in a "dry" mine where running or dripping water does not occur, the concentration and distribution of chemical obtained by this steeping method should give a fair degree of protection. It should be noted that the ends of freshly-cut mine timber would absorb preservative chemicals readily, affording extra protection at these most vulnerable points. There would be some penetration in the end truewood and a heavier concentration in the sapwood at the end than in the sapwood receiving the preservatives by side penetration only.

While it is impossible to make an accurate estimate, it seems to be a reasonable conclusion that green timber immersed for one week in 5 per cent. zinc chloride solution should give a service life of at least five years in a "dry" mine. Copper sulphate is probably more toxic to decay fungi in general than zinc chloride and is less subject to leaching, so it should be at least equally effective. Its main objection is its corroding effect on iron, but this should be of no importance in some classes of mine timbering under dry conditions.

It is important to note that, for guaranteed success with this type of treatment, the timber must be freshly cut and no surface drying should have occurred; otherwise, diffusion will be seriously retarded, and the penetrations and absorptions will be adversely affected.

## 7. Equipment for Treatment Under Commercial Conditions.

As the steeping treatment can be carried out at ordinary temperatures, no heating equipment is required. The most expensive item would be a vat which could be built of wood, or iron if zinc chloride were used, or possibly of concrete which would not be subject to any corrosive action by either chemical. It would be essential to add small quantities of some suitable fungicide to copper sulphate solutions to prevent mould growth.

### *Conclusion.*

Treatment of sapwood of green messmate (*E. obliqua*) by immersion in cold aqueous solutions of zinc chloride and copper sulphate gives penetrations and absorptions of the order required for preserving the wood under service conditions. A very high concentration (amounting probably to several pounds of chemical per cubic foot of treated wood) is rapidly built up in the outer layers of the sapwood (up to  $\frac{1}{4}$  inch in depth). For timbers requiring a short service life, it is probable that an immersion for one week in a cold 5 per cent. solution of zinc chloride or copper sulphate would give sufficient protection. If a greater service life is required, greater absorptions could be easily obtained by the use of longer immersion periods and more concentrated solutions.

TABLE 1.—SUMMARY OF RESULTS  $ZnCl_2$  DIFFUSION TREATMENTS.  
*Per cent.  $ZnCl_2$  present (on oven-dry wt. of wood).*

	20 per cent. Soln.				10 per cent. Soln.				5 per cent. Soln.				
	$0\frac{1}{4}''$ .	$\frac{1}{8}''-\frac{3}{8}''$ .	$\frac{3}{8}''-\frac{1}{2}''$ .	$\frac{1}{2}''-\frac{7}{8}''$ .	$\frac{7}{8}''-1\frac{1}{8}''$ .	$0\frac{1}{4}''$ .	$\frac{1}{8}''-\frac{3}{8}''$ .	$\frac{3}{8}''-\frac{1}{2}''$ .	$\frac{1}{2}''-\frac{7}{8}''$ .	$0\frac{1}{8}''$ .	$\frac{1}{8}''-\frac{3}{8}''$ .	$\frac{3}{8}''-\frac{1}{2}''$ .	
Four weeks' treatment	20.69	15.96	9.72	6.17	3.77	16.58	7.06	1.94	1.06	0.89	15.18	2.56	0.65
Two weeks' treatment	15.36	11.24	3.70	1.45	1.14	10.75	7.10	1.63	0.78	0.49	6.60	1.44	0.38
One week's treatment	13.77	9.39	1.95	0.59	0.47	12.87	3.72	0.46	0.24	0.25	10.46	0.74	0.45

TABLE 2.—SUMMARY OF RESULTS  $CuSO_4$  DIFFUSION TREATMENTS.  
*Per cent.  $CuSO_4 \cdot 5H_2O$  present (on oven-dry wt. of wood).*

	20 per cent. Soln.				10 per cent. Soln.				5 per cent. Soln.				
	$0\frac{1}{4}''$ .	$\frac{1}{8}''-\frac{1}{4}''$ .	$\frac{1}{2}''-\frac{3}{8}''$ .	$\frac{3}{8}''-\frac{1}{2}''$ .	$0\frac{1}{8}''$ .	$\frac{1}{8}''-\frac{3}{8}''$ .	$\frac{3}{8}''-\frac{1}{2}''$ .	$\frac{1}{2}''-\frac{7}{8}''$ .	$0\frac{1}{8}''$ .	$\frac{1}{8}''-\frac{3}{8}''$ .	$\frac{3}{8}''-\frac{1}{2}''$ .	$\frac{1}{2}''-1\frac{1}{8}''$ .	
Four weeks' treatment	19.3	9.1	4.2	3.1	23.5	7.9	5.1	3.3	2.2	19.5	3.4	1.2	1.01
Two weeks' treatment	24.0	2.9	0.90	0.81	20.0	2.0	0.57	0.31	0.22	5.8	1.07	0.24	Trace
One week's treatment	14.1	3.1	0.87	0.38	18.7	2.1	0.83	0.39	0.13	5.4	0.74	0.38	Slight trace.

# An Examination of Mulder's Rapid Biological Method for Estimating the Amount of Available Copper in Soils.

By A. M. Acock, B.A., D. Phil.\*

## Summary.

1. Mulder's proposal that the mould fungi may be used as the basis of a method of assessing copper availability in soils has been investigated.
2. It has been confirmed that the spore colour of *Aspergillus niger* grown on copper deficient media is dependent upon the amount of copper available to the fungus.
3. When soil suitably diluted with copper-free culture medium is used as the sole source of copper, the resultant spore colour of cultures of *Aspergillus* can be used as an indicator of the amount of copper in the soil that is available to the fungus.
4. It has been empirically established from a study of 46 Australian soils that, with appropriate quantities, soil "sound" in respect of copper can be differentiated from "unsound" soils, because the availability of copper to *Aspergillus niger* apparently parallels that to higher plants.
5. *Aspergillus niger* Neuberg (ex Lister 2380) has been selected as the most suitable of eight strains examined.
6. A procedure is recommended on the basis of experience with the method. This differs in several respects from Mulder's procedure, mainly in emphasizing the need for partial sterilization of soil before adding to cultures.

## I. Introduction.

Copper deficiency has recently been recognized as a handicap to the utilization of soils of certain types in many parts of the world. Reclaimed land in Holland, alkaline sands in South Australia, leached soils in Western Australia, and humus soils in Florida are examples [Mulder, 1938A, Marston *et al.* 1938, Teakle 1939, Allison 1930]. "Minor" element studies have special importance in Australia in the development of areas characterized by "coast disease" and "steely" wool in sheep, and by cereal deficiency diseases like "wither tip" [Strong 1941, Riceman and Donald 1938]. Detection of copper deficiency, apart from field tests and agricultural experience, has depended on tedious chemical and physical methods of analysis or else on biological tests of the Neubauer type. Analyses of whole soils or of soil extracts in hydrochloric acid, for example, may give little information about the availability of mineral nutrients to plants grown in the soil, and field tests and biological methods using higher plants are cumbersome. To overcome these obstacles to the rapid assessment of soil copper availability, attention has recently been directed to certain micro-organisms in which the degree of pigmentation is related to the amount of copper assimilated. Mulder (1938B) has made use of this fact to develop an extremely interesting biological method for copper

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assay in soils. The present piece of work was undertaken with the object of reviewing Mulder's claims and of seeing how far his method applies to Australian soils.

## 2. An Outline of Mulder's Research on Micro-organisms.

Mulder was interested in "reclamation" disease (Ontginningsziekte) in Dutch soils. He tested, on suitable media of carefully controlled copper content, a number of coloured micro-organisms, *Aspergillus niger*, *A. glaucus*, *A. flavus*, *Penicillium glaucum*, *Azotobacter chroococcum*, and manganese-oxidizing moulds; oxidation of ethyl alcohol to acetic acid by *Acetobacter aceti* was also studied. He found that spore colour of the moulds and oxidizing activity of the other micro-organisms as shown by pigmentation of the medium and acid production depended on the presence of traces of copper and could be quantitatively related to amounts of added copper ranging from zero to about 1 part in 10 million ( $5\gamma$  to 50 ml.). Certain strains of *Aspergillus niger* proved to have the most clear-cut response and were selected as the basis of further work. The most satisfactory strain of *Aspergillus niger*, grown on a balanced culture solution carefully freed of copper, formed after five days incubation a sterile white mycelium; with addition of  $0.2\gamma$  copper per 40 ml. of culture solution ( $\equiv 1$  part in 200 million), a vigorous sporing mycelium with orange-yellow spores was formed; with  $0.4\gamma$  copper, spore colour was light grey-brown; and with larger additions  $1.0\gamma$ ,  $1.8\gamma$  up to  $2.5\gamma$  copper (or 1 part in 16 million), brown, dark brown, and black spores were formed. This range of colours was repeatable and seemed to depend specifically on the concentration of copper, since a large number of other elements showed no comparable effects (Fe, Zn, Mn, Li, Ba, Ag, Pb, Al, Sn, Co, Ti, V, Mo, B, I, Cd). Particular emphasis must be laid on the question of the strain of organism used. Mulder examined five *Aspergillus niger* varieties from the collection of micro-organisms in the Laboratory of Mycology in Wageningen. The one finally selected (M) behaved as described above, two others (AG and Z) were similar except that they produced a vigorous crop of yellow spores by the fifth day of incubation on media with the lowest copper content practically obtainable; whereas NG and B formed yellowish brown mainly sterile mycelia without added copper, and greenish gray spores with no differentiation of colour with the addition of various amounts of copper.

Mulder then carried his investigation a stage further, applying his findings on *Aspergillus niger* (M) to the examination of copper availability in soils. The sensitivity of the mould to traces of copper is such that a tenth of a microgramme ( $0.1\gamma$ ) in 40 ml., or 1 part in 400 million, gives a detectable difference in spore colour, so that considerable dilution of soils is necessary to bring them within the sensitive range of the fungus. He found that, if 1 gramme of air-dry soil was added to 40 ml. of copper-free medium in a 1-litre Erlenmeyer flask and inoculated with the fungus, "sound" soils gave cultures with black spores whereas soils known to be agriculturally "sick" or "unsound" due to deficiency in available copper gave brown or yellow spores. He claimed further that by comparing the colour of cultures, where added soil was the copper source, with standard cultures of known copper content the amount of available copper in the soil could be quantitatively estimated.

### 3. Re-examination of Mulder's Claims.

As this re-examination of Mulder's technique has progressed, various modifications in the original procedure have been adopted for convenience and efficiency. Experimental work has concerned several aspects of the method, firstly as applied to standard cultures with known additions of copper sulphate, and secondly as applied to cultures supplied with soil as the only source of copper. Experimental findings are discussed under seven separate headings:—(i) Modifications in apparatus and quantities, (ii) selection of a suitable organism, (iii) spore suspensions and inoculation, (iv) spore colour studies and standards of comparison, (v) soil sterilization, (vi) application of the method to soils of known properties, and (vii) critical evaluation of the method. A final section is devoted to recommended procedures and precautions based on experience with the method.

#### (i) Modifications in Apparatus and Quantities.

Mulder used for his *Aspergillus* cultures 1-l. conical flasks of Jena glass plugged with cotton wool. He added 40 ml. of medium and 1 g. of soil. For convenience in incubating large numbers of cultures, 9-cm. Pyrex or Monax Petri dishes with lids have been used here, with addition of 20 ml. of medium and in soil studies 0·5 g. of soil. Liquid medium was used, purified from copper by boiling with calcium carbonate (see p. 298) before addition of minor elements. Details are given on page 298 of the washing of glassware to remove traces of adsorbed copper, the use of glass-distilled water, and the preparation of standard cultures of known copper content.

#### (ii) Selection of a Suitable Strain of Organism.

Attention was confined to the mould fungi, as Mulder found these to be the most satisfactory of the organisms he tested. Considerable emphasis was laid on the selection of a suitable strain. In all, eight strains of *Aspergillus niger* and one strain of *Penicillium glaucum* were examined. The *Penicillium* grown at 20°C. gave an excellent range of cultures from white through mottled green and white to full green with increasing amounts of copper; soils too were well differentiated, but the fungus is intolerant of temperatures of 25° to 30°C. Under Australian conditions, lower temperatures might be difficult to maintain, so attention was focussed on *Aspergillus niger* which is tolerant of higher temperatures. Media purified in several ways (calcium carbonate, ammonium hydrosulphide, and animal charcoal, and by the use of recrystallized salts of high purity, see p. 298) were used, but with none of the eight strains was it possible to repeat Mulder's finding of a sterile white mycelium on the fifth day of incubation at 30°C. with no added copper. Several strains showed a distinct range of spore colour, in response to increasing amounts of copper, from pale brown to black. The Neuberg strain (ex Lister 2380) was chosen for subsequent work, though not noticeably superior to the Wehmer strain (ex Lister 1692). Results with the eight varieties of *Aspergillus niger* are summarized in Table 1.

It is probable that other strains might be more sensitive to copper deficiency than those observed here.

TABLE 1.—SPORE COLOURS OF EIGHT *A. niger* STRAINS WITH TWO LEVELS OF COPPER.

Strain.	Range of Colour on Seventh day at 30°C.		
	0 Copper to 20 ml.		0·5 γ Copper to 20 ml.
1*	Grey-brown ..	..	Dark brown
2*	Irregular sporing with no definite range		
3*	Medium brown ..	..	Dark brown
4*	Dark greenish-brown ..	..	Dark brown
5†	Light greenish-brown ..	..	Medium brown
6	Poor irregular growth		
7 Wehmer (Lister 1692)‡	Light reddish-brown ..	..	Medium brown
8 Neuberg (Lister 2380)‡	Light reddish-brown ..	..	Medium brown

With 2 γ copper to 20 ml. all the strains gave very dark brown or black spores.

\* Obtained from Dr. E. Edwards, N.S.W. Dept. of Agric.

† Obtained from Dr. H. L. Jensen, Sydney University.

‡ Obtained from Prof. J. G. Wood, Adelaide University.

### (iii) Spore Suspensions and Inoculation.

The question of inoculation of the mould cultures raises some special problems. The normal black spores appear to contain some accumulation of copper (Steinberg 1935). It is desirable therefore that all plates for comparative purposes should have a uniform inoculum and as small a contamination of copper from the added spores as possible. But to ensure dense uniform cultures with a minimum risk of contamination from air-borne or surviving soil organisms, it is desirable to have a heavy inoculum of the required organism. To achieve these incompatible ends of small copper contamination and of dense growth, suspensions of brown spores from copper deficient cultures were found to be satisfactory (for details of the procedure finally adopted see p. 299). Markedly lighter cultures were obtained with brown spores than with black spore suspensions prepared in the same way.

### (iv) Spore Colour Studies and Standards of Comparison.

Of special importance is the colour of the mould spores as viewed in the aggregate on looking at the surface of a heavily sporing culture. With the aid of a Maxwell Disc Colour Analyser [see, e.g., Shaw 1932], composed of variable overlapping sectors of black, white, red, and yellow paper, it was possible to study quantitatively some important facts relating to spore colour. The colour standards used were those recommended by Hutton (1927) for soils supplied by the Munsell Colour Co., of Baltimore, Maryland, U.S.A., designated as follows [Schofield 1938] :—

TABLE 2.

Munsell's Number.	Rough Description.	C.I.E. (or I.C.I.) Co-ordinates.		
		X.	Y.	Z.
R 4/9 .. ..	Red	·2078	·1349	·0831
R 8/8 .. ..	Yellow	·5449	·5780	·1827
N 9/- .. ..	White	·7196	·7379	·8388
N 1/- .. ..	Black	·0196	·0200	·0236

These colours were found quite suitable for analysing the range of dull browns provided by copper deficient *Aspergillus niger* cultures. From the scale on the periphery of the disc, percentage composition of the colour is read in terms of the four components.

The colour of a series of five cultures of *Aspergillus niger* with 20 ml. of medium, 0, 0·2, 0·5, 1·0, and 2·0 microgrammes of added copper, and a standard inoculum of brown spores, incubated at 30°C., was examined systematically. For the first three days the mycelium was mainly sterile and white, but too discontinuous for a colour determination to be attempted. On subsequent days, despite some irregularity of the surface of the cultures and some patchiness of the colours, a fair match was possible by holding the cultures obliquely in a good light next to the spinning disc. The colour as measured in terms of percentage of black, white, yellow, and red was found to depend on two main factors, firstly the amount of available copper and secondly the age of the culture. This is illustrated in Fig. 1 in which the percentage of black in the colours of the five cultures with different amounts of copper is plotted against the age of the culture. It is clear that cultures darken with age, particularly till the seventh day, but that cultures with higher copper are consistently darker than those of the same age with a smaller amount. Mulder selected the fifth day of incubation for the examination of his cultures. It is apparent from Fig. 1 that, with the Neuberg strain studied here, the colours are darkening rapidly on the fifth day whereas by the seventh day they have become fairly stable. It would seem preferable to avoid the steep part of the curve by leaving final examination till the seventh day, especially as the cultures become more uniform over their whole surface as they grow older.

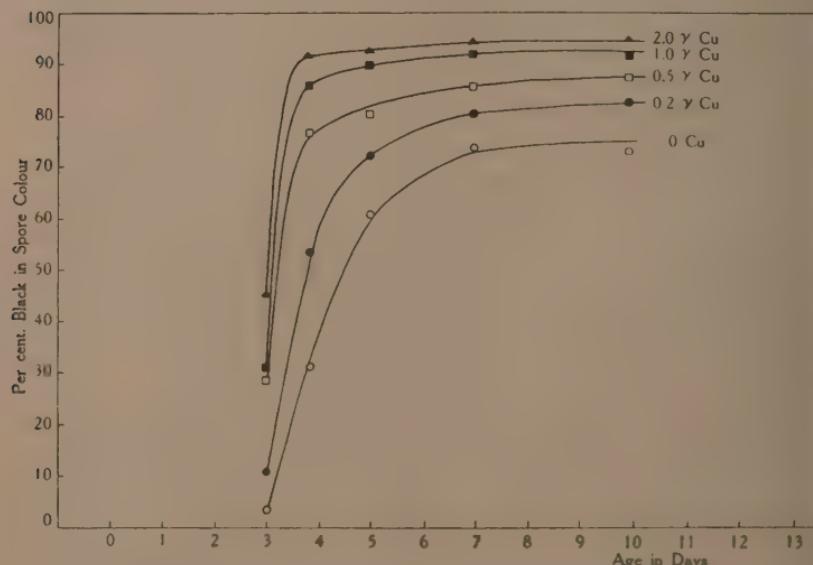


FIG. 1.—Showing the relationship between the proportion of black in the colour of *Aspergillus niger* cultures and the age of the cultures, with different quantities of added copper (in microgrammes per 20 ml.).

The black component in the colour was selected as showing the most marked trends. White behaves roughly inversely to black but falls to a very low percentage with all copper levels after the fifth day. Both the red and yellow components are higher in the case of cultures with low copper, red remains fairly steady from the third day onward, but yellow declines quite steeply from the third to the sixth day. The result of these colour trends is that cultures with low copper are consistently a paler more reddish brown than those with higher copper, where the proportion of black is higher.

The percentage composition of the colours of a typical range of standard cultures on the seventh day is given in Table 3.

TABLE 3.—PERCENTAGE OF COLOUR COMPONENTS IN THE COLOURS OF A RANGE OF STANDARD *A. niger* CULTURES SEVEN DAYS OLD.

Added Copper in Microgrammes ( $\gamma$ ).	Black *Neutral 1/-	White Neutral 9/-	Yellow Yellow 8/8.	Red Red 4/9.	Rough Description.
0	74	3·5	10·5	12·0	Dull reddish-brown
0·2	80·5	2·0	9·5	8	Dull reddish-brown, but darker
0·5	86·5	1·5	7·5	4·5	Dark grey-brown
1·0	92	1·0	4	3	Very dark brown
2·0	94·5	..	3	2·5	Very dark brown, almost black

\* These descriptions are those of the Munsell Colour Co.

It is clear from this quantitative study that spore colour trends form a fairly complicated picture. It does not seem advisable to use colour charts when attempting to assess the copper status of soils, but rather to grow standard cultures with known copper additions parallel with the cultures to which soil has been added.

#### (v) Soil Sterilization.

From preliminary tests on the addition of small amounts of soil to cultures, it was soon apparent that modification of Mulder's procedure of adding unsterilized air-dry soils is desirable. Serious contamination with more than one soil organism was encountered with three out of seven representative Australian soils added to cultures inoculated with *Aspergillus niger* and *Penicillium glaucum*. It is well known that autoclaving and steam sterilization of soils may increase the availability of soil nutrients [Russell 1932, p. 442], and that prolonged treatment with alcohol and acetone may render "sick" soils healthy [Mulder 1938A, p. 80]. Three of the above soils which gave the most serious contamination were saturated with a small volume of 70 per cent. alcohol for an hour, gently warmed to evaporate the alcohol, and then added to two series of cultures inoculated with *Aspergillus* and *Penicillium*. Untreated soils were added to two parallel series of cultures. After a week the cultures with unsterilized soil were all seriously contaminated with one or more organism, whereas only one *Aspergillus* culture and two *Penicillium* cultures with treated soil showed slight evidence of foreign organisms. Partial sterilization with

70 per cent, alcohol as detailed on p. 299 was adopted into the routine in all subsequent soil tests, and no serious contamination was observed in any subsequent tests of a wide variety of soils.

TABLE 4.—COMPARISON OF FIELD EVIDENCE REGARDING COPPER STATUS OF SOILS AND THE FINDINGS OF THE MULDER TEST.

Number of Sample.	Locality.	Field Reputation.	HCl Extractible Copper p.p.m. (Approx.) of Soil.	Copper Availability to <i>A. niger</i> in p.p.m. of Soil.
2606	Willalooka, S.A. ..	" UNSOUND SOILS "		
2167	King Island, Tas. ..	Steely wool ..		0·2
6088*	Kangaroo Island ..	Unsound ..	7·7 (H)	0·2
		Ataxia, puff pod condition in peas, responds to copper	8·0	0·5
6092	" " ..	"	22·0	0·5
6101	" " ..	" Probably unsound "	5·0	0·5
6102	" " ..	"	..	0·5
6121	" " ..	Very bad, peas show big response to copper	0·8	0·2
6122	" " ..	" " "	0·3	0·5
6124	" " ..	" " "	6·0	0·2
6128	" " ..	Very bad " .. "	1·0	0·2
6136	" " ..	Ataxia very bad ..	0·7	0·5
6154	" " ..	Acute symptoms of copper deficiency	0·3	0·2
6158	" " ..	Ataxia and failure of peas after a few years	1·0	0·2
6160	" Denmark, W.A. ..	" Unsound, " cobalt " deficiency recognized	0·4	0·2
3968	"	Unsound ..	6·6 (H)	0·4
3994	" Robe, S.A. ..	Unsound ..	5·9 (H)	0·4
5063	"	" Coast disease reported once	..	0·4
50	" Bews, S.A. ..	Unsound ..	..	0·5
..	Borrika, S.A. ..	Take-all in wheat and copper deficiency symptoms	..	0·5 to 1·0
..	Warrenben, S.A. ..	Steely wool, copper deficiency in plants	..	0·2
57	Corney Point, S.A. ..	Cu and Mn deficiency in wheat and oats	..	0·4 to 1·0
59	Lameroo, S.A. ..	Sheep show Cu deficiency symptoms	..	0·4
60	"	" "	..	0·4 to 1·0
70	" Pungonda, S.A. ..	Poor ..	..	0·2
68	"	Probably unsound ..	..	0·4
6176	Kangaroo Island ..	" DOUBTFUL SOILS "	7·0	0·4
6172	" " ..	Sugar gum country ..	7·0	1·0 to 1·5
		Has been handled successfully for crops and pasture but responds to Cu	..	
62	Warooka, S.A. ..	Adjacent to " coastal " area	..	0·4
54	"	"	..	1·0
85	Millicent, S.A. ..	Probably " unsound "	..	0·4

\* Samples from different horizons at the same site are bracketed.

TABLE 4.—*continued.*

Number of Sample.	Locality.	Field Reputation.	HCl Extractible Copper p.p.m. (Approx.) of Soil.	Copper Availability to <i>Aspergillus</i> <i>niger</i> in p.p.m. of Soil.
" SOUND SOILS "				
1439	Long Flat, S.A. ..	" Hospital area " for coasty stock	..	2 +
2220	King Island, Tas. ..	"	..	2 +
3496	Waite Inst., S.A. ..	Very good ..	20 (H)	2 +
3946	Denmark, W.A. ..	Sound ..	21 (H)	1.5 to 2
4527	Curlwaa, N.S.W. ..	Citrus, good ..	..	2 +
5942	Riddock, S.A. ..	Probably sound ..	..	2
8	Roseworthy, S.A. ..	Very good ..	..	2 +
14	Riverton, S.A. ..	Sound ..	..	2 +
17	Saddleworth, S.A. ..	" ..	..	2
29	Gulnare, S.A. ..	" ..	..	2 +
28	Georgetown, S.A. ..	" ..	..	2 +
43	Maitland, S.A. ..	" ..	..	0.4 to 1.0
49	Minlaton, S.A. ..	" ..	..	2 +
53	Yorketown, S.A. ..	" ..	..	2
84	Millicent, S.A. ..	" ..	..	1.5

(vi) *Application of Mulder's Method to Soils of Known Properties.*

In order to test the validity of the Mulder procedure as applied to soils, a range of Australian soils was examined from the Waite Institute's extensive stock of samples on which considerable field and laboratory evidence is available. Samples were included from Tasmania and Kangaroo Island, pastoral and wheat lands of South Australia, irrigated areas on the Murray, and from the Denmark estate in Western Australia. In the case of a set of Kangaroo Island soils, there are data\* on copper content from spectographic analyses of HCl extracts, and chemical data† are available on the HCl extractible copper from a few other soils‡ [Hosking and Burvill 1938]; 9 cm. Petri dishes with 20 ml. of medium and 0.5 gm. soil were used throughout. A range of standard cultures was included with every set of soils examined usually with 0, 0.1γ, 0.2γ, 0.5γ, and 1.0γ added copper. The amount of available copper in the soil in parts per million is determined by comparing the culture obtained by adding 0.5 g. of soil with the range of standards and multiplying by two the copper content, in microgrammes (γ) of added copper, of the standard culture most nearly matching.

The results obtained with 46 soils are summarized in Table 4. The soils have been grouped into three broad categories, "unsound," "doubtful," and "sound" on the basis of field evidence. It is clear that the evidence of the fungus method is in excellent general agreement with field observations. In the group of 27 unsound soils, copper availability readings are consistently at or below 1.0 parts per million, while the group of fifteen sound soils examined, with the exception of samples 3946, 43, and 84, had two or more p.p.m. of available copper. All the soils reported on in the table were examined in at least two separate sets of experiments. Results were consistently of the same order.

\* Oertel, A. C.—Unpublished. See Oertel, 1938, for an account of the method.

† Marked (H) in Table 4.

‡ Unpublished data by Hosking are included.

A fact that stands out is that the findings of the *Aspergillus* test are in better agreement with field evidence than the spectographic and chemical data obtained from hydrochloric extracts of soil. For example, soils 6088 and 6172 have the same order of acid extractible copper, but 6172 is superior both agriculturally and according to the Mulder test. In the case of 6092 (the subsoil of 6088) and 3496, acid-soluble copper is of about the same order, but 3496 is completely "sound" and gives black spored cultures whereas 6092 is "unsound" and gives brown cultures.

#### (vii) Critical Evaluation of the Mulder Test.

The Mulder test shares with other microbiological assay methods, e.g., for vitamins, the advantages of rapidity and reduced variability in the test organism over biological methods involving the use of large animals like rats, or of higher plants as in tests of the Neubauer type. Perhaps the most important claim made on behalf of the *Aspergillus* method of assessing the copper status of soils is that by using a plant as the basis of analysis the test provides a direct index of the availability of copper to plants, and hence possesses peculiar advantages over chemical and physical analyses of total soil or of acid extracts of soil. As has been seen, prolonged boiling with strong hydrochloric acid seems to extract considerably more copper from many soils than is available either to higher plants or to *Aspergillus niger*. But an exaggerated impression of close parallelism between the growth of *Aspergillus niger*, in liquid media to which small soil additions have been made, and that of a higher plant growing directly in the soil should be avoided. In the first place the fungus is strongly aerobic and does not grow in intimate contact with the soil, but as a superficial felt on the culture medium, so that its copper supply comes from the added soil by diffusion across a liquid path of about 2 mm. Secondly, many moulds are acid producers [Bennet-Clark 1937].

The Neuberg strain used here for example is a commercial citric acid producing strain. pH changes observed in 100 ml. of medium in 1-l. flasks with different copper additions are recorded in Table 5. There is a fall in pH for the first four days probably associated with acid excretion from the mycelium followed by a rise in pH presumably due to respiration of excreted acid and selective removal of  $\text{NO}_3^-$  from the potassium nitrate present, as the medium becomes exhausted. Soil reaction is known to influence ion availability considerably (Peech 1941). Many of the semi-arid and coastal Australian soils examined here are of a calcareous or solonized type, in which low copper availability may well be linked with pH effects, so it is possible that fluctuations in pH caused by the fungus may modify copper availability. A factor associated with the medium, which may also influence availability, is that potassium and calcium are added to the soil in concentrations that may release exchangeable copper.

Another loophole in the method is that a standard weight of soil (0.5 g. or 1.0 g.) is used in all cases, without allowance for differences in depth, texture, aeration, and other soil factors that may determine the volume of soil that is exploited by the roots of higher plants. A further sampling difficulty is that of obtaining truly representative and uniform samples of half or one gramme.

TABLE 5.—CHANGES IN pH IN 100 ML. CULTURES OF *Aspergillus niger*  
NEUBERG.

Days.	0 Copper.	5 γ Copper.	10 γ Copper.
0 .. .. ..	5·6*	5·6	5·6
2 .. .. ..	4·9	5·2	5·2
4 .. .. ..	5·3	5·4	5·4
6 .. .. ..	7·7	6·3	6·8
7 .. .. ..	8·0	7·7	7·4
9 .. .. ..	9·0	8·5	8·5

\* The medium was adjusted to approximately pH 6 with HCl.

Despite these theoretical objections, the data given here point to good empirical agreement with known copper availability in soils of a number of types. The absolute validity of the test can only be established on the basis of much wider application to soils, but it does appear to give at least a qualitative guide, and some quantitative evidence on the copper availability in soils. With the quantities advised here and in Mulder's papers, "sound" soils should give black or very dark brown spores, and only cultures with "unsound" soils should have brown spores coming within the sensitive range of the fungus. But there is no reason why the quantity of "sound" soil added should not be reduced to give cultures falling within the quantitative range of the standard cultures. By so doing the Mulder test may be extended as a quantitative index to cover both "sound" and "unsound" soils. It is doubtful, however, in view of the above objections, whether confidence should be placed in the quantitative value of the method applied to soils, although there is no doubt about the reproducibility and clarity of the results obtained with pure solutions of copper, and the extreme sensitivity of spore colour as an index of minute differences in the copper content of media.

The present findings are advanced to draw attention to a rapid and direct approach to the diagnosis of some minor element problems of widespread importance in Australia, and to emphasize certain modifications of the original Mulder procedure found from experience to be advisable.

#### 4. Recommended Methods and Precautions.

Details of the method finally evolved from this re-examination of Mulder's test, and of the precautions that should be observed are dealt with under a number of heads, covering the preparation of water, glassware, and media virtually free from copper, the preparation and inoculation of cultures of known copper content, and the testing of soils.

##### (i) Copper-free Water.

Double distilled water from a silica and Pyrex glass still was used for making up media and spore suspensions and for the final rinsing of all apparatus [Piper and Oertel 1941]. Any Pyrex or silica still with a good trap would be suitable. The still should be freed from

adsorbed heavy metals by distilling 0·25 per cent. acetic acid through it. Copper content of the water used here has been estimated to be of the order of 1 microgramme per litre.

### (ii) *Washing of Glassware.*

All apparatus must be made chemically clean by thorough washing with soap and water, strong chromate solution, or strong nitric acid. To remove adsorbed heavy metals, rinse with tap water, immerse in about 5 per cent. acetic acid in double glass-distilled water, and then rinse with double glass-distilled water.

For routine treatment of a large quantity of glassware (e.g., Petri dishes), a series of 2-l. glass troughs of strong nitric acid, acetic acid, and specially distilled water, with frequent changes of the water and acetic acid, was found satisfactory. The apparatus was drained on a glass plate and dried in an oven at about 150°C.

### (iii) *Media.*

Several methods are available for the preparation of liquid media practically free from heavy metals. The various constituents may be individually purified [Piper 1940]. Mulder precipitated heavy metal contamination in the whole medium as sulphide. He recommended a culture solution of the following composition:—

Double glass-distilled water ..	1,000	ml.
Glucose .. .. ..	50	g.
KNO <sub>3</sub> .. .. ..	5	g.
K <sub>2</sub> HPO <sub>4</sub> .. .. ..	2½	g.
MgSO <sub>4</sub> .7H <sub>2</sub> O .. .. ..	1	g.

and supplementary heavy metals, added after purification—

FeCl <sub>3</sub> .6H <sub>2</sub> O .. ..	50	mg.
ZnSO <sub>4</sub> .7H <sub>2</sub> O .. ..	20	mg.
MnSO <sub>4</sub> .4H <sub>2</sub> O .. ..	3	mg.

The purest commercial preparations were used. As these still contained small amounts of copper,  $\frac{1}{3}$  ml. of a solution of NH<sub>4</sub>HS (ammonium hydrosulphide) was added, and then  $\frac{1}{2}$  per cent. (5 g. per l.) of norit (animal charcoal). After five minutes shaking the solution was filtered cold. Iron, zinc, and manganese were added after this treatment, molybdenum was not considered necessary. Mulder's medium was tried here with success, but much seems to depend on the quality of animal charcoal available. An alternative method used by Steinberg (1935) and also by Stout and Arnon (1939) is precipitation of heavy metal traces in the whole medium (except for supplementary heavy metals) by boiling with calcium carbonate and filtering. This has been adopted in the present work: 10 g. of pure CaCO<sub>3</sub> per litre was added, the solution vigorously boiled with shaking for five minutes, cooled overnight, and the carbonate filtered off. Medium of the same composition as Mulder's was used, except that ferric citrate equivalent in iron content to 50 mg. ferric chloride per litre was substituted, and a trace of sodium molybdate added. For the supplementary minor element solution, the purest obtainable salts were dissolved in glass-distilled water. Media prepared in this way are not entirely free from copper, but are easily made and low enough in copper to give *Aspergillus niger* Neuberg cultures with pale brown spores. Media were adjusted to pH 6 with 1N HCl.

(iv) *Standard Cultures.*

The copper solution for making up standard cultures of known copper content was prepared in two strengths:

- (i) of 10 microgrammes ( $\gamma$ ) copper per ml., i.e., 39.2 mg.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  per litre.
- (ii) of 1 microgramme ( $\gamma$ ) copper per ml., i.e., 3.92 mg.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  per litre (prepared by dilution).

A 1-ml. graduated pipette freed from copper was used for adding measured quantities of each solution. The more dilute solution was used for quantities of 1 microgramme and less. A suitable range of cultures of known copper content for comparison with a set of cultures with 0.5 g. of added soil is prepared by adding 0, 0.1 $\gamma$ , 0.2 $\gamma$ , 0.5 $\gamma$ , 1.0 $\gamma$ , and 2.0 $\gamma$  copper, equivalent to 0, 0.2, 0.4, 1.0, 2.0, and 4.0 parts of available copper per million parts of soil.

Nine-cm. Pyrex (or Mouax) Petri dishes were washed and heat-sterilized, then 20 ml. of medium added from a small measuring cylinder, copper additions made, the plates rotated to ensure uniform distribution of copper, inoculated with *Aspergillus niger* Neuberg (ex Lister 2380), and incubated at 30°C. 750-ml. Pyrex conical flasks with cotton wool plugs may be used with 20 ml. of medium (and 0.5 g. of soil), or, as Mulder recommended, 1-l. conical flasks with 40 ml. of medium (and 1.0 g. of soil).

(v) *Inoculation.*

Stock cultures were grown on agar slopes with Czapek-Dox medium. The selected strain of *Aspergillus niger* was subcultured into 20 ml. of copper-deficient liquid medium in a Petri dish, incubated for 7 days at 30°C. Twelve "loops" of brown spores from the aerial growth were removed with a platinum wire washed off and kept suspended by shaking vigorously in 20 ml. of copper-free water in a small conical flask. 0.5 ml. of this spore suspension, added from a pipette plugged with cotton wool and well distributed, should give dense uniform inoculation over the whole surface of a Petri dish culture. Old standard cultures deficient in copper are a good source of copper-deficient spores. The spores retain their viability for a long time.

All subculturing, pouring of plates, and inoculating was done observing bacteriological precautions, in a still dust-free atmosphere.

(vi) *Soil Samples and Soil Sterilization.*

Care should be taken in selecting representative sites for the collection of soil samples. The half-gramme samples examined here were taken at random from bulk samples collected from the top six inches, or at several levels in a profile and passed through a 2-mm. stainless steel or aluminium sieve after breaking up aggregates with an iron pestle and mortar. The method is so simple, however, that 10 or more half-gramme samples might be taken from the top six inches of an area with a cork borer or cheese sampler and investigated separately. Finely divided air-dry soil (0.5 g.) was transferred to a sterile acid-washed Petri dish, moistened with 0.5 ml. of 70 per cent. alcohol, covered for one hour, then the lid displaced and the dish warmed to 30°C. to drive off the alcohol, 20 ml. of medium added, and 0.5 ml. of spore suspension.

Special care should be taken to spread the soil evenly over the bottom of the dish to avoid patchy cultures. This was done with a flattened copper-free glass rod after pouring and inoculating the medium.

#### (vii) Interpretation of Soil Tests.

Cultures with soil additions and standards with known copper additions are incubated together at 30°C. for seven days and the soil cultures then compared with the standards. As a qualitative guide to decide whether a soil is likely to be copper-deficient or not, a single standard might suffice, such as a culture with 0.5 g. of a soil known to be sound in respect of copper, or else a culture with 1.0γ or more copper per ml. From Table 4 it appears that soils with 2 parts per million or more of available copper will probably be "sound", those with less, giving brown-spored cultures, are likely to be copper-deficient.

#### 5. Acknowledgments.

My thanks are due to Prof. J. A. Prescott, Chief of the Division of Soils, for suggesting this piece of work, and to my colleagues of the Waite Institute for their interest and assistance.

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# The Efficiency of Phenothiazine Against *Nematodirus* spp. in Sheep.

By G. P. Kauzal, Dr.Vet.Med.\*

## Summary.

Phenothiazine in a single dose of 0.6 g. per kilo of body weight was highly effective as an anthelmintic against *Nematodirus* in sheep. Some residual infestation remained, but this was reduced still further by a second dose of 1.2 g. phenothiazine per kilo body weight. A new device was used to detect fluctuations in faecal egg counts when the number of eggs per gramme of faeces is low.

## 1. Introduction.

Although proof of the pathogenicity of species of *Nematodirus* for sheep is lacking, it is believed that the very large numbers frequently found in the small intestine may, at times, have important effects on the general health and nutrition of the host. Kauzal (1933) recorded 32,000 *Nematodirus* in one lamb, and of many sheep examined during the same period, more were infected with *Nematodirus* than with any other species of parasite. Ewer (1938), quoting observations made in New Zealand in 1935, refers to mean counts of 3,760 *Nematodirus* in fat sheep and 11,530 in sheep which were not thriving. Lapage (1940) found 50,000 *Nematodirus* in a lamb about four months old.

Ryksen (1939) records that *Nematodirus* species are the only worms commonly found in sheep grazing on the Great Karroo of South Africa. When seasonal conditions necessitate holding these sheep on small areas for hand-feeding, heavy infestations soon build up and losses occur. The affected sheep show emaciation, weakness, and pallor of the mucous membranes, but diarrhoea is absent.

Studies concerning the pathogenicity of these species for sheep, which are in progress, have yielded no tangible evidence of pathogenicity as yet, but the facts just quoted are sufficient warrant for an experiment to determine the effect of phenothiazine on species of *Nematodirus* affecting sheep in Australia.

As Britton (1941) points out, the efficiency of phenothiazine in expelling *Nematodirus* from sheep has varied widely in the hands of different workers. Habermann and Harwood (1940) found that 25 g. of phenothiazine administered to a sheep weighing 70 lb. removed only three out of a total *Nematodirus* population of 318. Swales (1940) recorded a mean efficiency of 72 per cent, with doses ranging from 19.0 to 46.5 g., given in tablet form. Taylor and Sanderson (1940) found that phenothiazine in tablet form in doses of 10 to 30 g. was ineffective against *Nematodirus* in goats, but that doses of 15 g. were 56 per cent. effective in two somewhat heavily infested lambs. They considered *Nematodirus* among the most resistant to phenothiazine of the trichostringylid worms inhabiting the small intestine.

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## 2. Experimental Procedure.

Faecal egg counts were used as a measure of the results of treatment. Since *Nematodirus* are poor egg-layers, it was necessary to devise a method for determining small numbers of eggs per gramme of faeces accurately. A special slide was devised for this purpose by Mr. H. Whitlock, Senior Assistant in the Parasitology Department of this laboratory. This slide, and the manner of its use, is described elsewhere (Whitlock, 1941). It will suffice to say here that it permits the eggs in 3·5 ml. of a suspension containing 3 g. faeces in 60 ml. of 50 per cent. saturated solution of sodium chloride, to be concentrated by flotation and counted. The method was thoroughly tested and gave accurate results.

Twelve ewes were selected, each of which was passing reasonably numerous *Nematodirus* eggs in the faeces. Regular faecal examinations were made for several days before and after the administration of phenothiazine. The phenothiazine was administered in the form of a suspension. The dose for each sheep was weighed separately, shaken in water, and administered immediately by drenching. The ewes were grouped as follows:—

*Group A*.—Three ewes, each of which received a single dose of phenothiazine at the rate of 0·6 g. per kilo body weight.

*Group B*.—Three ewes, each of which received 0·6 g. per kilo body weight, and a second dose eleven days later of 1·2 g. per kilo body weight.

*Group C*.—Six untreated ewes which served as controls.

The twelve ewes were kept in the same pen and shared the same rations throughout the trial.

## 3. Results.

The mean egg counts per gramme of faeces for the individual sheep up to the date on which treatment was administered were:—Group A—17, 207, 86; Group B—61, 20, 69; and Group C—23, 49, 40, 36, 27, 171. But for the special technique and egg-counting slides already mentioned, fluctuation in these small counts would not have been detectable.

Following the first dose of phenothiazine, the six treated sheep in Groups A and B showed reductions in the faecal egg count, ranging from 47·7 per cent. to 96·4 per cent. In only one instance was the reduction less than 80 per cent. During this period the faecal egg counts of the six control sheep decreased, but whereas in one control sheep the reduction was 84·4 per cent., it ranged from nil to 43·0 per cent. in the others.

No reason can be given for the decreases in egg counts among the control group during this period, but since all the sheep were penned and fed together the same factors probably had some influence on the egg counts of the treated sheep also.

Following the second treatment of Group B, the residual infestations were further reduced by 77·4 per cent., 79·0 per cent. and 91·6 per cent. in the three sheep respectively. It was noted, moreover, that during this period the infestations among the control sheep tended to increase slightly while those of Group A remained unchanged.

#### 4. Conclusions.

Where losses from *Nematodirus* infestation occur, or are suspected, a single dose of phenothiazine at the rate of 0·6 per kilo should be sufficient to reduce the infestation to a relatively insignificant level.

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# A Useful Mixing Apparatus for the Preparation of Suspensions of Faeces for Helminthological Examinations.

By G. P. Kauzal, Dr.Vet.Med.,\* and H. McL. Gordon, B.V.Sc.\*

## Introduction.

With the extension of investigations to large groups of sheep in the field, the necessity arose for increasing the ease and speed of preparing faecal egg counts and cultures. During the past two years an average of over 200 egg counts and cultures have been prepared weekly and have occupied much time. The egg-counting technique developed at this laboratory (Gordon and Whitlock, 1939)† definitely increased the ease and speed of carrying out faecal examinations. In the preparation of the faecal suspensions, however, the shaking of each specimen with steel beads, the pouring of each into and out of the shaker, and the washing of the shaker, beads and cork were involved. To prepare faecal cultures, individual faecal samples had to be mixed with finely-ground, dry, helminthologically-sterile sheep faeces, either by stirring with a spatula in a small jar or by rubbing and crumbling in the hands. Both methods were time absorbing.

## The New Mixing Apparatus.

A mixing apparatus (Fig 1) has been designed and consists of an electric hand-drill fitted with mixing blades on a spindle. The spindle passes through a brass bearing mounted in a rubber stopper. The stopper fits tightly into the jars in which the faecal suspensions are prepared.

Faecal suspensions are prepared in batches of three, as the special counting chamber slides devised by Whitlock† consist of three chambers, and samples are then withdrawn and introduced into the counting chambers. The spindle and blades are cleansed between the mixing of each suspension by running the motor with the spindle immersed in running water.

It has been found that from 5 to 8 seconds is sufficient for the preparation of a thoroughly mixed faecal suspension.

To prepare faecal cultures, the faecal sample with some dried, finely-ground, sterile sheep faeces is placed in a small jar (see Fig. 1), the stirring spindle is introduced so that the rubber stopper fits tightly into the opening of the jar and the motor is run for 2 to 3 seconds. If the mixing is continued for a longer period the faecal sample is apt to be ground too finely and, when water is added, it forms a compact mass which does not favour optimal development of eggs and larvae. The faecal culture medium should be a lightly crumbled, spongy mixture with spaces between faecal particles to provide aeration.

\* An officer of the Council's McMaster Animal Health Laboratory, Sydney.

† Gordon H. McL., and Whitlock, H. V. (1939).—*J. Coun. Sci. Ind. Res. (Aust.)*, 12: 50.

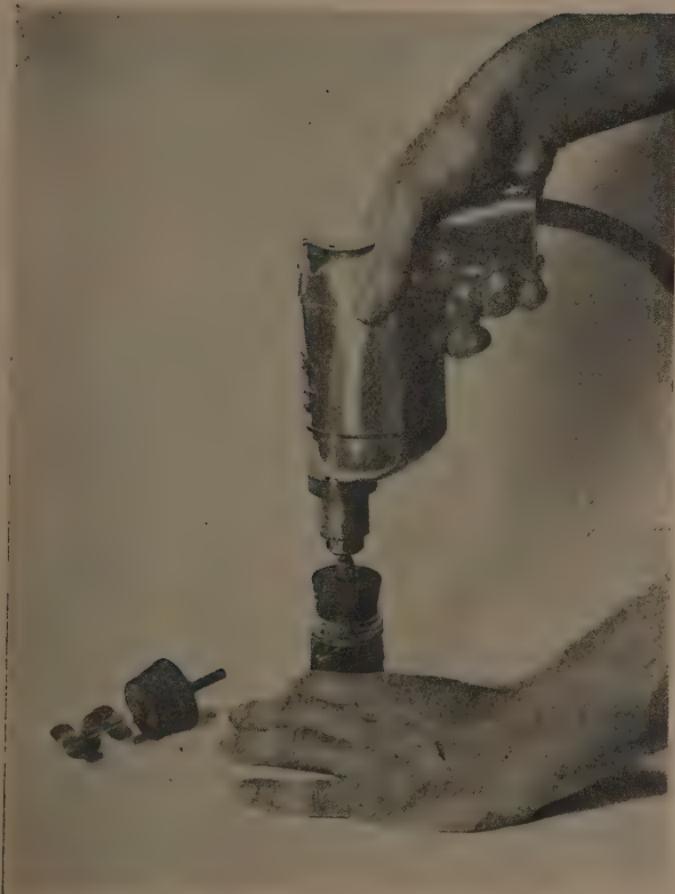


FIG. 1.

The faecal suspensions prepared for egg counting at this laboratory contain saturated sodium chloride solution as the flotation fluid. It is impossible to avoid some splashing of this fluid on the motor-housing of the mixing apparatus. It is necessary, therefore, to clean the motor-housing repeatedly and to apply liberal quantities of vaseline, or to have the housing painted with a salt-resistant paint (duco spraying is satisfactory). Even when these precautions are taken, certain parts of the apparatus are likely to corrode. Constant cleaning and care will reduce this to a minimum.

This apparatus has reduced the time taken to prepare faecal suspensions and cultures to about one-quarter of that previously required and does the mixing more efficiently.

It can be used for many purposes that involve mixing, e.g., the preparation of small samples of emulsions.

# A New Apparatus for Counting Small Numbers of Nematode Eggs in Faeces.

By H. V. Whitlock.\*

## Summary.

An apparatus which permits the accurate estimation of small numbers of nematode eggs in faeces has been devised. Its preparation and use are described.

The apparatus and technique here described have been used for several months in studies on the infestation of sheep with *Nematodirus* species (Kauzal, 1941). Since these parasites produce very few eggs compared with many of the nematodes infesting sheep, the egg-counting technique in common use at this Laboratory (Gordon and Whitlock, 1939) was unsuitable for the special studies. In infestations with *Nematodirus* spp., the number of eggs per gramme of faeces is frequently less than 200, which is the minimum that can be counted by the usual method. It was necessary, therefore, to concentrate eggs from a larger bulk of faeces and yet to avoid too unwieldy a volume of fluid. The apparatus described here permits the estimation of as few as six eggs per gramme of faeces. The principle of the method is to concentrate the eggs by flotation in a chamber of known volume and then to slide away for examination its shallow top portion which contains the eggs.

## Description of the Apparatus.

The apparatus is shown diagrammatically in Fig. 1. It is made from strips of celluloid 1 mm. thick, cemented together by means of acetone.

The flotation chamber consists of a rectangular trough 2 cm. square and 6.5 mm. deep, over which a sliding top 2 cm. square, with three walls 2 mm. deep, can be moved. The roof of the sliding top is preferably made of glass cemented to celluloid strips by Canada balsam and is engraved on its lower surface with crossed lines which divide it into four equal areas for convenient counting. A celluloid roof readily becomes scratched and cloudy. The walls of the apparatus extend 3 mm. above the level of the platform. This prevents the overflow of faecal suspension and guides the sliding top.

When the sliding top is placed over the trough, it completes a chamber 2 cm. square and 8.5 mm. deep with a narrow horizontal opening towards the platform. The chamber as described holds 3.5 ml., but dimensions can be varied and multiplying factors computed accordingly.

## Preparation of Faecal Suspension.

Three grammes of faeces are thoroughly soaked in 30 ml. of water, and 30 ml. of saturated sodium chloride solution are then added. The mixture is shaken vigorously with glass beads or small steel ball-bearings till an even suspension is obtained. The electric mixing apparatus described by Kauzal and Gordon (1941) will give this in about five seconds.

\* An officer of the Council's McMaster Animal Health Laboratory, Sydney.

### Technique.

The sliding top is moved over the trough with its open side adjacent to the platform. The chamber thus formed is filled by means of a wide-mouthed pipette with faecal suspension immediately after mixing. To avoid bubbles on the under-surface of the sliding top the apparatus is tilted slightly while the flotation chamber is filled.

The apparatus is then set aside on a level surface under a bell jar to retard evaporation in hot, dry weather. From 30 to 60 minutes are allowed for the nematode eggs to rise and settle on the lower surface of the sliding top. The apparatus is then held firmly by the base, and the point of a dissecting needle is inserted between the back of the sliding top and the end of the body of the apparatus; the sliding top is slid gently across to the platform, and carries with it the thin layer of relatively clear fluid containing the nematode eggs.

The apparatus is then transferred to the stage of a microscope and the eggs are counted. With the apparatus and faecal suspension here described, the number of eggs counted, when multiplied by the factor 5·7, gives the estimated number of eggs per gramme of faeces.

### References.

Gordon, H. McL., and Whitlock, H. V. (1939).—*J. Coun. Sci. Ind. Res. (Aust.)*, **12**: 50.  
 Kauzal, G. P. (1941).—This *Journal*, p. 301.  
 Kauzal, G. P., and Gordon, H. McL. (1941).—This *Journal*, p. 304.

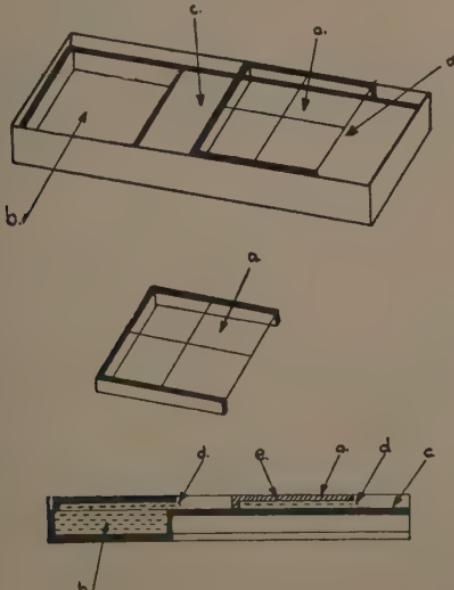


FIG. 1.—Diagrammatic representation of the counting apparatus. The top figure is drawn in perspective and shows the sliding top in position for microscopic examination. The centre figure shows the sliding top removed: *a* the sliding top; *b* the rectangular chamber; *c* the platform; *d* the open side of the sliding top; *e* the under surface of the sliding top.

## Note on an Experimental Kiln for the Smoke-curing of Fish.

*By E. W. Hicks, B.A., B.Sc.,\* M. C. Taylor, M.Sc.,\* and  
E. J. Ferguson Wood, B.A., M.Sc.†*

A considerable quantity of fish is smoke-cured in Australia at the present time, and it is probable that this method of preservation would be more widely used if means could be found of overcoming some of the difficulties in producing smoked fish of uniformly good quality at a reasonable cost. The smoke houses in use at present are generally of the traditional Scottish type, and the methods of operation are similar to those in use in Scotland. However, it seems that in all but the most southerly parts of Australia, the atmospheric temperatures and humidities are too high, for a considerable part of the year, to obtain optimum conditions for smoking fish by the Scottish methods, and even the most painstaking curers find it almost impossible to obtain uniformly good results.

The Divisions of Food Preservation and Fisheries have, for some time, been considering the problems confronting curers. A great deal of experimental work on the smoke-curing of fish has been carried out at the Torry Research Station, Aberdeen, and by the Fisheries Research Board of Canada, but it was considered that further work with Australian fish under Australian conditions was necessary. Consequently a specially designed experimental kiln has been constructed, and a series of experiments with it was begun in February 1941. The principal objects of this work are:—

- (1) To define optimum smoking conditions for the more important species of fish available in Australia, and to determine upper limits of kiln temperature and humidity for satisfactory working.
- (2) To explore likely methods of counteracting unfavourable weather conditions particularly—
  - (a) increasing the rate of evaporation from the fish by using relatively high air velocities;
  - (b) extending and accelerating the preliminary drying of the fish before subjecting them to smoke-laden air;
  - (c) the use of artificial dehumidification.

These experiments are far from complete so that final conclusions cannot yet be given. It has, however, been established that temperatures and humidities considerably higher than those recommended for Scottish and Canadian fish may safely be used with Australian mullet.

The layout of the experimental equipment is represented diagrammatically in elevation in Fig. 1, and the layout of the duct system is shown in Fig. 2.

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\* An officer of the Division of Food Preservation and Transport.

† An officer of the Division of Fisheries.

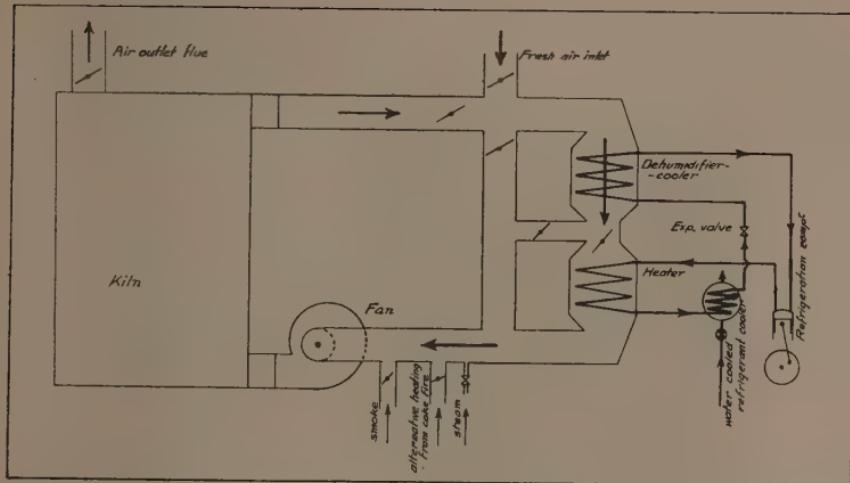


FIG. 1.—Experimental smoke-kiln using heat-pump cycle for dehumidification.



FIG. 2.—Duct System of Kiln. A = Outlet duct from kiln. B = Kiln proper. C = Inlet duct. D = Humidity measuring equipment. E = Return duct. F = Dampers. F1 = Recirculation damper. F2 = Air inlet damper. F3 = Smoke damper. F4 = Auxiliary heat damper. F5 = Dehumidifier by-pass damper. F6 = Reheater by-pass damper. F7 = Reheater damper. G = Fresh air intake. H = Smoke box. I = Inlet duct to fan. K = Finned coil dehumidifier. L = Finned coil reheater. M = 4-ton compressor. N = Auxiliary heater. O = Steam valve to increase humidity.

This apparatus is designed to give any desired conditions in the air stream independent of outside air conditions, and it is not intended to suggest that a similar design is desirable in commercial plants. A forced air circulation with provision for varying amounts of recirculation is used. A small ammonia refrigeration unit is used for cooling and dehumidifying the air when necessary. To obtain a sufficient degree of dehumidification, it is generally necessary to cool the air well below the temperature desired. Reheating is therefore necessary, and this is done by circulating hot gases from the compressor discharge through a finned coil in the air stream before they pass to the water cooled condenser, i.e., the heat pump principle is applied. This gives a considerable saving in running costs over other methods of reheating. To obtain heating without smoke in cool weather, air is drawn over a coke fire fitted under a branch duct from the main air stream. Provision is also made for the injection of steam to raise humidities when desired.

# The Commercial Production of Fish Liver Oils.

By E. J. Ferguson Wood, B.A., M.Sc., A.A.C.I.\*

## 1. Introduction.

For some twelve months the Fisheries Division has been studying the possibilities of production of fish liver oils in Australia. The foundation of a shark liver oil industry has already been reported,† but, as this does not supply the needs of the poultry industry, it is considered that a general description of the methods used for the extraction of fish liver oils would not be out of place.

## 2. Design of Plant.

The apparatus that is shown in the photographs (Plate 4) is quite effective for quantities up to 1 cwt. of livers a day—treated in two batches. It consists of a stainless steel digester, 15 inches wide, 20 inches deep in the cylindrical section with a conical section 15 inches deep. This is connected to a plug cock by means of 2-in. pipe, and below the plug cock are a sight glass, a 2-in. T piece, and a second plug cock for draining the stick-water. The side arm leads from the T piece through a third plug cock to the centrifuge. Steam is admitted to the digester through an open coil in the conical section, the coil being  $\frac{1}{2}$  inch diam. pipe with  $1/16$  inch holes. In practice it is found necessary to place a round  $\frac{1}{4}$  inch mesh wire strainer in the digester to hold back large particles, and a fine screen is used over the centrifuge. This could be improved by having a bowl over the centrifuge with a sloping screen and a 2 inch drain pipe at the foot of the screen. The oil-water emulsion would flow to the centrifuge from below the screen and would be admitted above it.

A paddle stirrer driven by a motor is used to stir the mass. The motor has a rubber bung on the spindle and this drives a horizontal brass disc connected directly to the paddle. This gives a friction clutch and an infinitely variable speed.

The centrifuge in use is a De Laval 120, but some prefer the Sharples super-centrifuge with its higher centrifugal speed. By installing a second digester, the centrifuge could run almost continuously while the digesters were heated in turn. Steam is obtained from a 5 H.P. colonial boiler which is also used for several other purposes.

## 3. Methods of Extraction.

The methods used for extraction of fish liver oils may be divided into—

- (i) steam extraction,
- (ii) alkaline extraction,
- (iii) oil solvent extraction,
- (iv) digestion, and
- (v) solvent extraction.

\* An officer of the Division of Fisheries.

† *Asian J. Pharm.* n.s. 22, (259): 473, 1941.

(i) *Steam extraction.*

This is the simplest method, but can only be used for livers such as shark and cod which break up easily and in which oil is abundant to the order of 30 to 50 per cent. of liver weight. In this method the minced liver, with hot water added, is boiled with live steam for a period of 30 minutes or so, until the liver tissue has disintegrated. The oil comes to the surface, the stock-water is run off through the drain cock, and the oil centrifuged together with the upper aqueous layer. For oils that are not required for medicinal purposes, skimming is sufficient, but vitamin oils require rapid treatment to prevent the oxidation of vitamin A. Therefore centrifuging is advisable.

This method is suitable for livers of sharks, rays, swordfish, &c.

(ii) *Alkaline extraction.*

A number of patents are in existence for the extraction of fish liver oils by alkaline digestion. Several of these were tried, but the most successful method does not appear to be covered by patents in Australia. This method consists essentially of digesting one volume of minced livers and two volumes of water by means of the open steam coil. The mixture is adjusted with caustic soda to pH 7.8 to 8.0. If the livers prove refractory, the pH may be raised slightly, but this also increases saponification of the oils. Yellowtail and mullet gut oils obtained by this process can be quite golden yellow in colour, though alkaline digestion is reported to darken the oil. The period of digestion is of the order of 30 minutes at 95°C. The mass should be stirred continuously and is then centrifuged.

This method can be applied to yellowtail, flathead, mullet, salmon, striped tuna, and barracouta. It is not effective for bluefin tuna (*Thunnus maccoyii*).

(iii) *Oil solvent extraction.*

A method applicable only to livers high in vitamin, notably to bluefin tuna. It is usually combined with the soda process which is allowed to proceed normally except for the addition of a quantity of oil, preferably a low vitamin oil such as salmon body oil. Centrifuging recovers the added oil which has dissolved the original liver oil and the vitamins. At times the vitamins do not seem to be all in solution in livers of very low oil content, but, according to certain authorities, are associated with proteins. An oil solvent soda digestion is therefore required to extract these vitamins. A solvent extract of the raw liver is necessary as a laboratory test to estimate the potency of the liver so that the amount of oil to be added may be judged.

(iv) *Digestion.*

This method was crudely practised in the rotting of cod livers to obtain the oil. In order to avoid high acid values and other deterioration, the natural enzymes are prevented from working either by arranging the pH or by heating. Papain, pepsin, and trypsin are used. These methods have been tried with southern bluefin tuna but were not markedly successful. The oil-in-oil method (section 3 (iii)) was found to be more suitable.

(v) *Volatile solvent extraction.*

Two methods are available, firstly using a heated solvent in an extractor of the Soxhlet type, and secondly a cold solvent extraction. Cold solvent extraction has been used commercially and there are patents for certain solvents used in this connexion. The cold extraction method is expensive and slow and shows no advantages over the soda, and oil solvent methods. Solvents used include petroleum ether and ethyl acetate. The minced livers are dried with twice their weight of sodium sulphate and then extracted for a time up to three weeks with the solvent. At the end of this period the solvent is filtered off, the residue washed with fresh solvent and the original filtrate and the washings distilled off leaving the oil. Only high potency oils can be payably extracted in this way.

#### 4. Extraction of Australian Fish Livers.

The fish oil research programme of the Fisheries Division is by no means complete, and this is to be regarded as a progress report which is felt to be opportune.

(i) *Shark Livers*.—These can be digested by steam alone, and can be readily treated by a plant similar in essentials to the pilot plant depicted in the photograph.

(ii) *Salmon Livers (Arripis trutta)*.—From preliminary results it seems doubtful whether it is more payable to extract these separately or to add them to the fish meal plant. The reason for this is that they do not appear on preliminary assays to possess high potency oil, the yield is not high, and the livers small. The salmon body oil produced by the Narooma Canning Co. has a rather low vitamin content for animal feeding, but such an oil is very valuable at the present time as a solvent for higher potency oils. For example, it could profitably be used for extracting bluefin tuna livers. It is stated that livers should be extracted by fish oils rather than by vegetable oils.

(iii) *Southern Bluefin Tuna (Thunnus maccoyii)*.—The use of salmon body oil for extraction has already been mentioned. Striped tuna livers mixed with bluefin livers yield a semi-solid blackish waxy oil; and barracouta guts may also be used to produce a solvent oil. It should be recorded here that the pyloric caecae of bluefin and striped tunas yield more oil than do the livers, but the vitamin content of these oils is not yet known. The recommendation would be therefore—in New South Wales extract bluefin tuna liver oils with salmon body oil, and in Tasmania, mix the bluefin livers with striped tuna livers or barracouta guts. It is difficult to suggest any alternative, as a large bulk of low grade fish oil is unobtainable.

(iv) *Striped Tuna (Katsuwonus pelamis)*.—Soda digestion is adequate for these livers.

(v) *Barracouta (Thyrsites atun)*.—The livers are small and rather hard to extract. The gut yields 6 to 8 per cent. oil of a dark colour. Its vitamin content is not yet known.

(vi) *Mullet (Mugil dobula)*.—The livers are too small to be profitably extracted, but the gut yields from 6 to 16 per cent. of a light yellow waxy oil, by the soda process.

(vii) *Flathead* (*Platycephalus* spp.).—The yield of oil is low and the vitamin content too low for the livers to be extracted profitably. The same applies to the gut, as far as this fish has been tested. Both soda process and ether extraction were used.

(viii) *Yellowtail* (*Kingfish of N.S.W.*) (*Seriola grandis*).—This fish has been mentioned in a note in the *Journal*,\* and even higher recoveries of oil—up to 36 per cent., have been obtained by ether extraction, and 16 per cent. by soda extraction.

### 5. Conclusion.

The present position is that any large increase in the production of vitamin-rich oils for stock feeding can only occur if the fishing industry in general is enlarged. To this end, the salmon and especially the tuna canning industry may be expected to contribute.

The question of using mullet gut oils as a source of stock-feeding oils may be summed up on the following basis. Assume an annual marketing on the east coast of Australia of 2,000 tons of fish. This would give approximately 100 tons of guts, producing an average of say 10 per cent. of oil=10 tons, or 2,500 gallons of oil, not a very large yield. It does not seem therefore that the shortage of fish oils for animal feeding can be economically relieved by recovering oil from market offal. With the normal abundance of green food and sunlight in Australia, the shortage for stock feeding will possibly result in most cases merely in a falling off of quality, and for poultry feeding in the quantity of eggs produced, but does not seem to threaten a cessation or even a shortage of supply. The establishment of a tuna canning industry on a firm and permanent basis would completely restore the fish-oil position.

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\* This *Journal*, 14: 69, 1941.

## The Harvesting of *Gracilaria confervoides* for Agar Agar.

By E. J. Ferguson Wood, B.A., M.Sc.,\* and H. M. Peddie.\*

In continuation of the work on agar agar referred to in a previous issue of this *Journal*,† the question of a continuous supply of the raw material arose.

Although it has been established that large quantities of *Gracilaria confervoides* are washed up on certain beaches at times, it is quite possible that an industry might find itself running short of raw material owing to seasonal or other variations. It is known that the weed occurs in a number of situations on the New South Wales coast, at various depths up to 20 fathoms, so it would seem that the flotsam harvest could be supplemented by harvesting from the sea bed.

For this reason attention has been devoted to mechanical harvesting, and various methods were tried including a net with a chain on the bottom, a rake, and so forth. From experience gained in these experiments, the harvester described below was designed and built.

The principle of the harvester is that of the Agassiz trawl, in fact a 5-ft. Agassiz trawl was used in the first tests, one rope of the trawl being wired to a 2 in. by  $\frac{1}{2}$  in. iron bar to which were riveted on its upper side a number of triangular blades made for horse-drawn mowing machines. The trawl was dragged by a length of four-strand 3-in. manila rope, the blades having a tearing as well as a cutting action. Running against the tide, the weed was cut and caught in the bag of the net, but a large harvest of sand showed that modifications were required. These consisted in altering the position of the cutter bar and in substituting a 2-in. mesh net for the  $\frac{1}{2}$ -in. mesh of the Agassiz trawl.

The present trawl consists of two D-shaped bows with two spacer bars halfway up the straight and curved limbs of the D. The net is attached to the four rear corners and wired to the cutter bar which is bolted across the base of the bows. Rings are attached to the back of the net with a draw rope to allow of ease in emptying a heavy catch. A glass buoy on a light line is attached to the trawl net and acts as a direction indicator and also as a salvage line in case the tow rope parts, or the trawl fouls. A bridle is attached to the front of the D about 4 inches above the front cross bar. A section of chain is lashed around the top sector of the bow to allow of adjustment of the position of the bridle and the cut of the harvester is set by means of this adjustment and by the length of rope used for towing. The position of the cutter bar can be altered within limits by bolt holes drilled through the base of the D bows. A refinement might be to use a bow with the lower portion serrate, and a cutter bar bolted down with a D bolt diagonal to cutter and bow. The trawl is represented diagrammatically in Fig. 1 and a photograph of it is given as Plate 3, Fig. 1.

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\* An officer of the Division of Fisheries.

† This *Journal*, 14: 221-2, 1941.

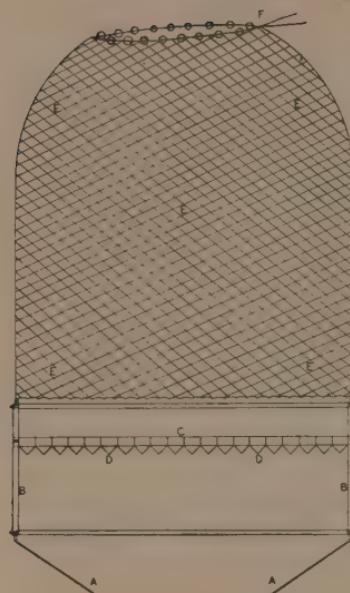


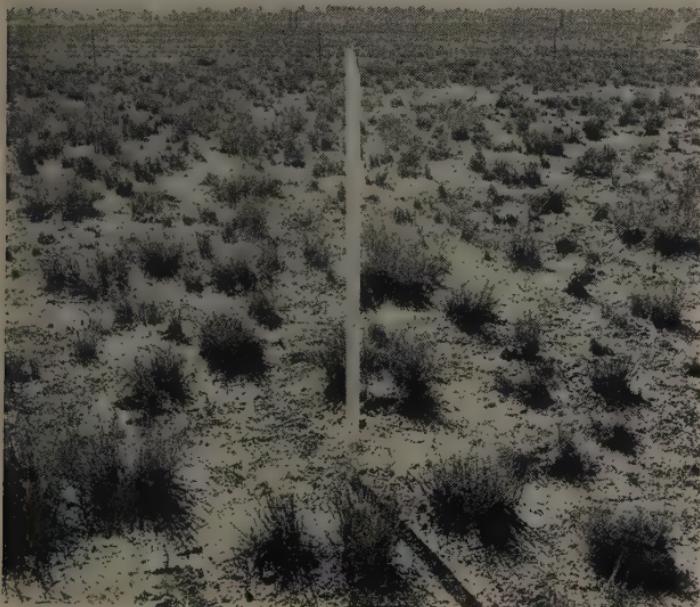
FIG. 1.—Top view of trawl harvester:  
A, bridle; B, frame; C, cutter  
bar; D, blades rivetted to  
cutter bar; E, 2-in. mesh net;  
F, "cod-rope."

The harvester has been towed by a 25-ft. boat fitted with a single cylinder 7 H.P. engine. The best towing speed was about quarter-speed, and fast towing leads to a decreased yield. There was little difference between tows with and against the tide. A five minute haul at Port Hacking resulted in about 70 lb. of *Gracilaria* with little sand, but the weed here is rather sparse. At Botany Bay four hauls of 4 minutes produced 600 lb. of wet seaweed representing 1 hour's work, and at Bateman's Bay, three hauls of 5 minutes produced about 400 lb. representing half an hour's work. Such a harvester would be suitable for harvesting in shallow areas (6 to 100 ft. of water) from small boats, such boats having preferably a cockpit and a mast from which a boom and tackle could be used to hoist the machine inboard. A cruiser or compromise stern is preferable to a counter. Larger vessels operating in deeper waters would use an 8 or 10 ft. trawl, and would need a boom and winch.

An 18-in. harvester (Plate 3, Fig. 2) has been made for sampling areas that cannot be inspected by eye or with a water telescope. It is hoped by this means to extend our knowledge of the *Gracilaria* beds in New South Wales' waters, and later to extend the tests wherever a suitable launch is available. This trawl is effective using a boat powered with a  $2\frac{1}{2}$  or 3 H.P. engine, and to a tested depth of about 15 fathoms.

**PLATE 1.**

(Studies on the Mitchell Grass Association in South-western Queensland. See p. 253.)

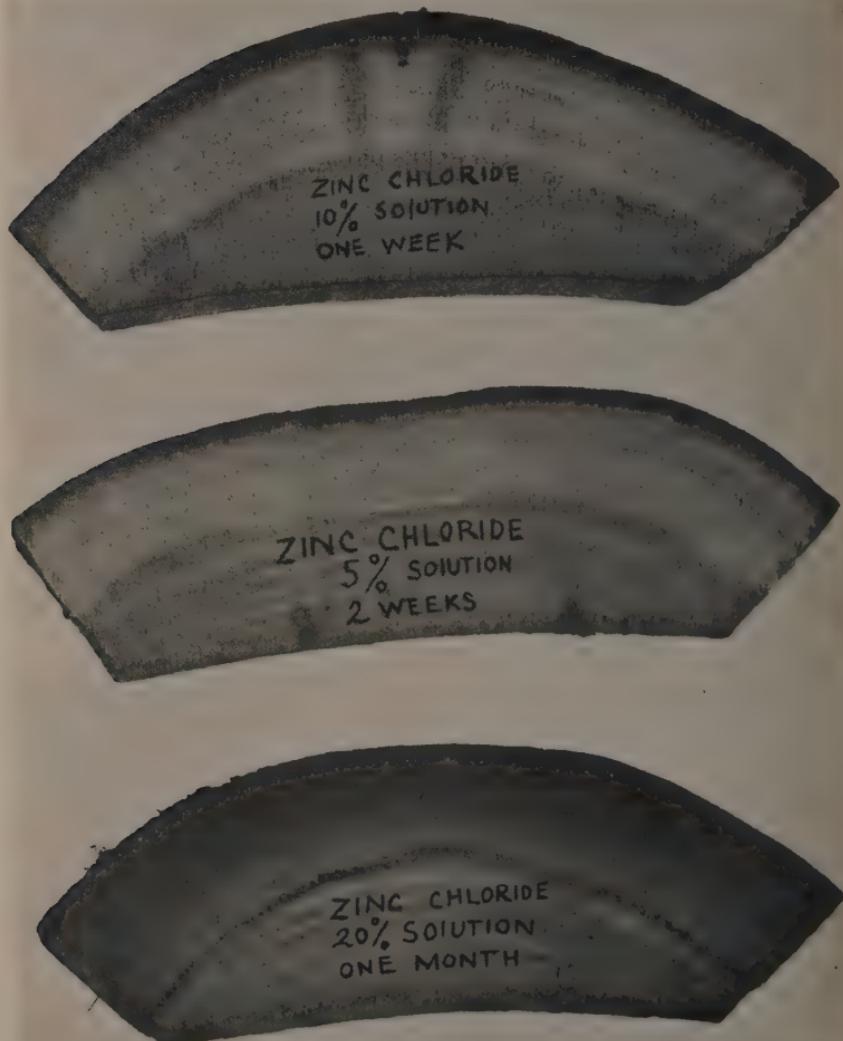


**FIG. 1 (above).**—Mitchell grass pasture at Gilruth Plains, November 1940. Mitchell grass plants consist of short dry stubble no other species present.

**FIG. 2 (below).**—The same pasture in February 1941. Mitchell grass seeding. Good growth of annual species.

PLATE 2.

(A Quantitative Investigation of the Steeping Method for the Preservation of Timber. A Modern Application of an Old Process to Australian Mine Timbers. See p. 281.)



**PLATE 3.**

(The Harvesting of *Gracilaria confervoides* for Agar Agar. See p. 315.)

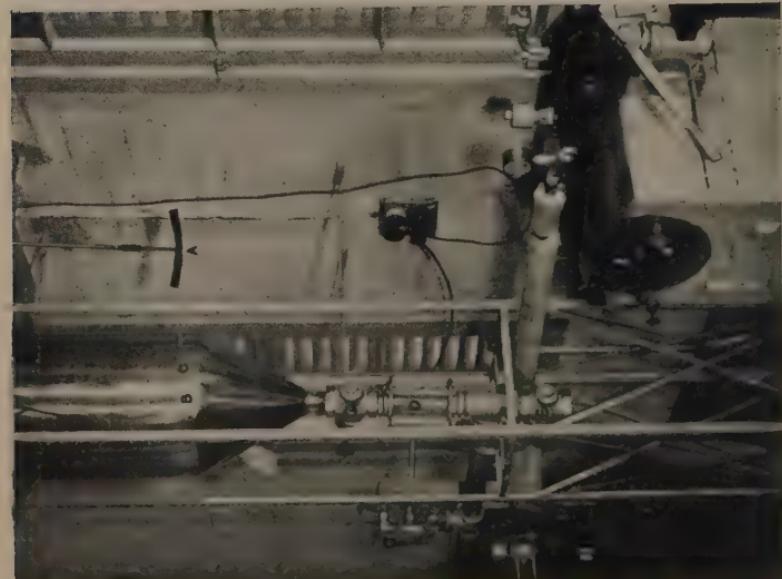
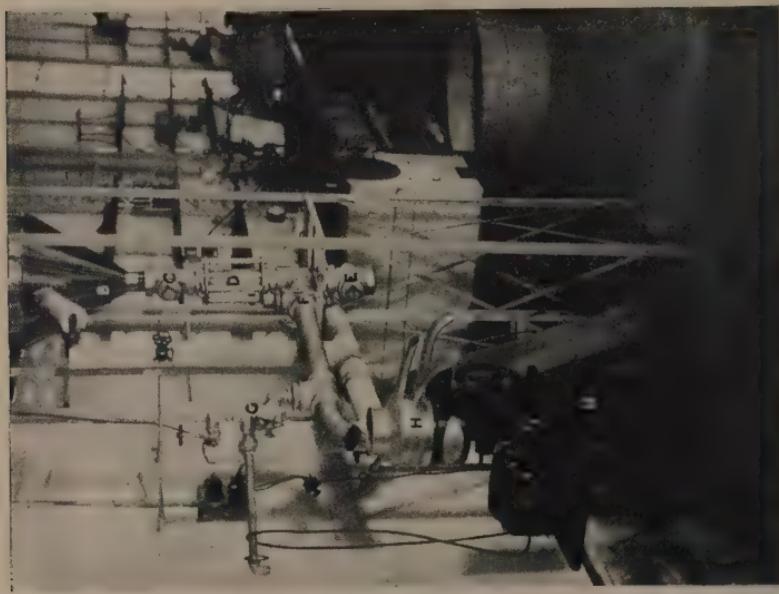


FIG. 1 (above).—Trawl harvester.

FIG. 2 (below).—Sampling trawl.

PLATE 4.

(The Commercial Production of Fish Liver Oils. See p. 311.)



Fish liver oil investigation pilot plant at the Council's Fisheries Division Laboratories, Cronulla.  
A, paddle stirrer ; B, digester ; C, digester plug cook ; D, sight glass ; E, drain cock ; F, outlet cook to centrifuge ; G, hot water ejector and steam inlet ; H, centrifuge ; I, small boiler used for digestion in original installation.

## NOTES.

### B.T.B. 15 Blowfly Dressing.

This blowfly dressing was described by F. G. Lennox in an article entitled "Some Experiences in the Preparation of Blowfly Dressings and a description of a New Boric Acid Mixture" (this *Journal* 14: 1, May 1941). The dressing has since been tested under field conditions and has proved very satisfactory. It is being recommended by officers engaged in extension work on the blowfly problem.

Considerable difficulties have arisen over supplies of the special tar oil recommended by Lennox because it is needed for war industries. A suitable substitute has therefore been sought. Several tar oils have been examined but none has been found which, when used alone, can satisfactorily replace the special tar oil. The function of tar oil in the dressing is twofold: (a) to act as a contact poison—this effect is relatively slight but is of great importance in preventing maggots from crawling to adjacent areas and establishing themselves there; (b) to enable the dressing to wet and penetrate the fleece easily—this is an essential property of all good dressings. Tar oils that have a similar B.P. (boiling point) range nevertheless vary greatly as regards (b), and hence satisfactory tar oils can only be selected by suitable tests. Their B.P. ranges and broad specifications do not indicate their merit for use in blowfly dressings.

Two tar oils have been examined which, when mixed with equal parts of power kerosene, have proved highly satisfactory under laboratory and insectary conditions, and the dressings made with them are as effective as the original B.T.B.: their ability to wet and penetrate the fleece is greater than that of the original B.T.B. The two oils that have been found effective are: "Creosote 259" and "Middle oil 43," both of which can be obtained from Timbroil Pty. Ltd., 32 Walker-street, Rhodes, Sydney. These tar oils are obtainable in commercial quantities and are unlikely to be demanded for war purposes. Power kerosene is a relatively standardised product and can be procured from the usual sources.

There may be other tar oils available in Australia which would serve as well, but it must be reiterated that proper tests are required in each case. Their suitability cannot be taken for granted. Tests on sheep are time consuming and costly and it is suggested that, for the time being at any rate, manufacturers should use one or other of the two oils here mentioned.

The prescription for B.T.B. 15 includes 2 per cent. of tar oil. This can be replaced by 2 per cent. of a 50/50 mixture of power kerosene with one of the two specified tar oils. When the tar oil and power kerosene are mixed, a slight deposit forms. This can be discarded after it has settled out.

Although these substitutes for the special tar oil have not been tested under field conditions, tests on artificially induced strikes in sheep have been entirely satisfactory, and the modified formula is expected to give just as good results under field conditions as the original.

As all samples of bentonite have not proved equally satisfactory, manufacturers are advised to use one of the bentonites of American origin, supplies of which are available either from Messrs. Berks Products, Sydney, or from The Standard Chemical Co., Melbourne.

Since Lennox's description was published, B.T.B. 15 containing 15 per cent. of boric acid, and B.T.B. 30 containing 30 per cent. of boric acid, have been found to give very similar results under field conditions. The lower concentration of boric acid is therefore recommended.

### Economy of Lubricating Oil—A Note on the By-Pass Filtration of Crank Case Oil.

*(Contributed by L. T. Wilson, B.Sc., A.A.C.I.\*)*

It is common practice in the operation of motor vehicles to drain and discard the lubricating oil from the sump after each 1,000 miles of running. In some cases the period is extended to 2,000 miles. The oil is discarded because of the accumulation in it of suspended carbon, dust, metal fragments, &c., and to chemical deterioration in the oil itself. This chemical deterioration, which is largely an oxidation, leads to an increase in acidity, to polymerization and the formation of "gumming" products. The suspended material may be removed by filtration, but the acids and polymerized products which are in solution must be removed by absorption or by chemical methods.

The capacity of the oil sump in a car or truck is of the order of two gallons, so that after the vehicle had completed 10,000 miles the amount of oil used (apart from any "make-up" oil) would be about 20 gallons. Various proprietary oil cleaners which may be fitted to the vehicle have been advertised. These consist of a filter through which a proportion of the oil is by-passed during the running of the engine. The makers of these cleaners claim that the original oil in the sump may then be used for about 10,000 miles without changing.

We have examined one of these cleaners. It consisted essentially of a tightly packed cotton waste filter. The makers state that this is chemically treated in some way, but it is probable that the main action is one of simple filtration. The filter is so designed that when it becomes clogged it may be readily removed and a new cartridge inserted.

The proprietors of a motor service were interested in using these filters and asked us to report on the condition of the sump oil after it had been used for an extended period. The oil from the sump was drained by the proprietors and sent to us for analysis.

#### *Results.*

Four samples have been examined. Two of these had been in use for 10,000 miles and the other two for 15,000 miles. The engines were 1940 models, 27 h.p., and were operating in passenger buses carrying a total load of about 6 tons including tare. These vehicles have a

\* An officer of the Council's Lubricants and Bearings Section, which is accommodated in the Chemistry School of the University of Melbourne.

relatively small sump, and the amount of original oil necessary to fill it plus the filter was approximately  $1\frac{1}{4}$  gallons. At the beginning of the test, the engines were practically new and were in good condition. The filtering cartridge was not changed throughout each test. The results were as follows:—

Oil Sample No.	Oil Mileage.	Total Make-up Oil.	Ash.	Petroleum Ether Insoluble.	Acid Value.	Viscosity.	Dilution.
		qts.	Per Cent.	Per Cent.	mg. KOH/g.	secs. R1 at 140°F.	Per Cent.
1	10,000	2	.01	Negligible	1.2	227	0.36
2	10,452	1	.01	..	1.1	210	0.0
3	15,021	7*	.01	..	0.7	220	..
4	15,329	6½*	.005	..	0.7	223	1.15
Original Oil SAE 30	..	..	Negligible	..	0.7	190	..

\* The comparatively large amount of make-up oil used is attributed by the proprietors to overfilling. This extra addition would of course help to maintain the sump oil in good condition.

It will be seen that in all cases the oil is in good condition. The increase in viscosity is not great and the amount of suspended solids is negligible. The amount of dilution is small. The increase in viscosity indicates that a certain amount of soluble decomposition products has been formed. The oil was still clear in appearance but it showed a progressive darkening in colour, being very dark after 15,000 miles. The low ash together with the moderate acid value suggests that little corrosive wear is occurring and that the bearings are not likely to be affected.

The above analysis does not include an estimation of the soluble decomposition products. If the concentration of these products becomes too great, it leads to gumming and to valve and ring sticking. The operators of the vehicles report that there was in general no sign of gumming or ring sticking. The vehicle on No. 4 test (15,000 miles) was pulled down and was found to be in good condition, except that one valve showed some indication of sticking. Those results suggest that, under these operating conditions, a life of 15,000 miles is perhaps the maximum that could be expected before the accumulation of the soluble products would cause trouble. It should be noted that these vehicles were in a practically new condition and were operated by experienced drivers. With engines which were more worn, the strain on the filters would be correspondingly heavier and, under certain conditions, greater dilution might occur.

#### Discussion.

These results suggest that a very considerable economy of oil may be effected by the use of a simple filter of this general type. The oil consumption per 10,000 miles may, for example, be reduced from 20 gallons (1,000 miles change) or 10 gallons (2,000 miles change) to 2 gallons, and under present circumstances such an economy would be of considerable importance in Australia.

These filters are of course not new and their existence and performance is known to many technical people; nevertheless, it may be worth considering whether they should not be used much more extensively in this time of emergency and fitted to commercial and

private vehicles. They may also prove valuable when fitted to producer gas vehicles, since they will remove *in situ* the dust and abrasive particles which cause increased wear.

It is emphasized that no exhaustive examination of the performance of these filters under different conditions of operation has been made. The observations have so far been limited to those described in this note. The object of the publication is to draw general attention to the possibilities of a simple device of this kind.

At the present time all the sump oil that is drained from vehicles is not discarded. A certain proportion of it is collected and, after physical and chemical treatment, used for various purposes. It is also not uncommon for many of the larger transport organizations to employ a stationary filtering plant of their own which prolongs the life of the oil. Under certain conditions, particularly in metropolitan areas where the used oil can be more readily collected, reclamation may be the most economic method. In country districts it may prove easier to use either stationary filters or filters fitted to the vehicles.

#### New Designs for Boxes—Saving in Money and Materials.

One of the first war-time tasks of the Division of Forest Products was to suggest suitable Australian timbers for substitution in various specifications and in some instances to suggest modification of the specifications, especially where unnecessarily high quality was called for. Among many such specifications were those covering various types of cases for the transport of munitions of different types. These were taken in hand comparatively recently, when the greatly increased demands on timber made it necessary to conserve supplies as much as possible, and a programme of re-design investigation was commenced. The aim was to produce cases at least equally as strong as those in use but to reduce the amount of timber required per unit.

The investigations to date have yielded astonishing results. In the first two modified specifications to be accepted for service uses, on one contract alone there was a saving of £35,000 in cost and of 750,000 super. feet of timber. Proposed modifications of other cases are awaiting final approval and, if accepted, will result in savings of approximately ten times the above amount. Still others are approaching a state where they can be put into use.

Thus, this investigation alone, when complete, will result in enormous savings of timber and thus help to eke out supplies. It will also result in a direct saving to the Government of far more money than has been spent on the whole Division since its inception.

#### Index to Horticultural Abstracts Volumes I-X.

The 10-year Subject and Author Index to Horticultural Abstracts 1931-1940 has been printed; it was scheduled to appear on 20th September.

The price will be not less than 25s. sterling, and there will be no free issue. All orders should be sent direct to:

Imperial Agricultural Bureaux,  
Central Sales Branch,  
Agricultural Research Building,  
Penglais, Aberystwyth, Wales.

### Industrial Research in the United States.

The activities of the National Resources Planning Board of the United States cover a very wide field. The latest publication of the Board is a report on industrial research in America; the survey was carried out by a committee formed of members of the National Research Council assisted by representatives of industrial laboratories, universities, and research institutions.

The report emphasizes the way in which the application of science in industry is contributing to the high standard of living in the United States. It is estimated that the 70,000 research workers employed by American industry in some 2,200 laboratories, represent an annual expenditure of \$300,000,000. Industrial research acts as a protection against unfavourable changes taking place both within and without an industry, and the report recommends that leaders in several industries take steps towards initiating research programmes where they do not now exist. The amounts spent on research in various industries vary widely, but the chemical industry is the one devoting the greatest percentage of its income to research.

It is of interest to note that although industry looks to the universities for trained technical men and for the principal advances on the frontiers of science, such advances are not infrequently made in the course of research projects originally designed to achieve immediate commercial objectives.

In discussing the question of abstracts of the technical literature, the report points out that in several branches of pure and applied science the work of abstracting is supported by scientific societies. Owing to the enormous and rapidly expanding amount of technical matter being published, such support is becoming increasingly burdensome and increasingly inadequate. The National Resources Planning Board considers that an excellent means of Government contribution to industry would be proper provision for systematic and complete publication of abstracts of scientific and technical literature.

The Board also points out that extension of research means increasing dependence upon adequate and correct standards of reference. Highly trained technical workers, expensive facilities, and exacting and prolonged laboratory work are necessary for the establishment of standards, and it is recommended that encouragement and increased monetary support be given to the National Bureau of Standards, so that more research may be carried out on standards of measurement. Ample provision should also be made for adequate publication and distribution of the Bureau's findings.

In transmitting the report to President Roosevelt, the Board drew attention to the great importance of industrial research in both the present defence effort and also to developments in the post-defence period.

### Recent Publications of the Council.

Since the last issue of the *Journal*, the following publications of the Council have been issued:—

*Bulletin No. 140.—“Foot-Rot in Sheep. A Transmissible Disease Due to Infection with *Fusiformis nodosus* (n.sp.). Studies of its Cause, Epidemiology, and Control,”* by W. I. B. Beveridge, D.V.Sc.

This Bulletin shows that foot-rot can be completely eradicated from a flock by examining the feet of all sheep in the summer and removing all carriers of infection for treatment or slaughter.

Foot-rot is a contagious disease and cannot arise spontaneously on wet pastures. Thus it should not be confused with the non-contagious foot infections that follow injury or long exposure to wet mud; these latter infections are usually confined to one foot on an animal, and usually to one digit, and are mostly found in adult sheep, whereas foot-rot attacks young animals readily, and almost invariably affects both digits on the one foot and often more than one foot.

It has been found that the infecting organism in foot-rot can live for only a few days away from the sheep's foot, so that if an infected yard or pasture is spelled for a fortnight there is no chance of it still being contaminated. Under the conditions usually prevailing in Australia, the infection survives during the dry summers on the feet of some members of the flock, but not in the soil.

Treatment of affected animals consists in paring the hoof to expose all pockets of infection and immersing the foot for about ten seconds in a solution of 30 per cent. copper sulphate or 10 per cent. formalin.

*Bulletin No. 141.—“A Soil Survey of the Waikerie Irrigation Area, South Australia,”* by R. I. Herriot, B.Ag.Sc., and E. J. Johnston, B.Sc.Agr.

In 1927, the Council's Division of Soils began a study of the soils of the irrigated horticultural settlements in the Murray Valley. The object of the work was to obtain a detailed knowledge of the soils of these high-priced lands, as it is only in the light of such knowledge that the best methods of utilizing the soils and maintaining their fertility can be developed. Up to date, 22 settlements extending from Murrabit, Victoria, to Mypolonga, South Australia, have been covered by soil surveys. With the publication of the present Bulletin, all except a few relatively small, privately owned, areas have now been completed.

The survey at Waikerie covered about 6,000 acres. The soils are closely related to those found in many other irrigation settlements along the River Murray; they belong for the most part to the Mallee zonal group. About two-thirds of the area is devoted to vines, and most of the remainder is used for citrus growing. The Bulletin discusses the various crops grown and their relationship to soil types and drainage practices. Zinc-deficiency diseases seem prevalent in certain areas but are likely to be controlled by appropriate zinc treatments.

The distribution of the soil types is shown on a coloured soil map of the area.

*Pamphlet No. 108.—“Studies on Some Ectoparasites of Sheep and their Control. 1. Observations on the Bionomics of the Sheep Ked (*Melophagus ovinus*),”* by N. P. H. Graham, B.V.Sc., and K. L Taylor,

B.Agr.Sc. "2. Chemical and Biological Studies on Certain Arsenical Dipping Fluids," by M. R. Freney, B.Sc., M. Lipson, B.Sc., and N. P. H. Graham, B.V.Sc. "3. Chemical Observations on Commercial Powder Sheep Dips with Special Reference to their Arsenic Content," by M. Lipson, B.Sc.

These studies were undertaken as a means of evolving a more efficient dipping routine. In studying the life history of the sheep ked or tick, it was found that keds live for 80 to 100 days when on the sheep but will survive for only a few days off the animal. They migrate readily from sheep to sheep, but the susceptibility of different sheep to infestation varies very widely; there are indications that younger sheep and those in poor condition are more susceptible. Keds deposit their pupae on the wool fibre some distance from the skin of the sheep, the greatest proportion being deposited on the neck and brisket, so that they can all be removed at shearing if these areas are properly cleaned up.

Experiments were undertaken to compare the toxicity or poisoning power of a number of different arsenical compounds used for dipping, in order to find the most suitable dip to use as a standard in further trials; sodium arsenite was found to be the most satisfactory compound tested. Experiments also showed that there was little decrease in the arsenic concentration of the dipping fluid after a large number of sheep had passed through it.

#### Forthcoming Publications of the Council.

At the present time the following future publications of the Council are in the press:—

*Bulletin No. 142.—"A Soil and Land Use Survey of the Hundreds of Riddoch, Hindmarsh, Grey, Young, and Nangwarry, County Grey, South Australia,"* by C. G. Stephens, M.Sc., A.A.C.I., R. L. Crocker, M.Sc., B. Butler, B.Agr.Sc., and R. Smith, B.Agr.Sc.

*Bulletin No. 143.—"Production of Dried Grapes in Murray Valley Irrigation Settlements. 1. Viticulture,"* by A. V. Lyon, M.Agr.Sc., and D. V. Walters, M.Agr.Sc.

*Bulletin No. ?.—"Enzootic Ataxia and Copper Deficiency of Sheep in Western Australia,"* by H. W. Bennetts, D.V.Sc., and A. B. Beck, M.Sc.

*Bulletin No. ?.—"An Analysis of the Outbreaks of the Australian Plague Locust (*Chortoicetes terminifera* Walk.) during the Seasons 1937-38 and 1938-39,"* by K. H. L. Key, M.Sc., Ph.D.

*Bulletin No. ?.—"Friction and Lubrication Report No. 1.—The Theory of Metallic Friction,"* by F. P. Bowden, Sc.D. (Cantab.), and D. Tabor, Ph.D. (Cantab.).

*Bulletin No. ?.—"Studies in Fertility in Sheep. II. Seminal Changes Affecting Fertility in Rams,"* by R. M. C. Gunn, D.V.Sc., B.Sc.Agric., M.R.C.V.S., R. N. Sanders, B.V.Sc., and W. Granger, B.V.Sc.

*Bulletin No. ?.—"Interference in a Wind-Tunnel of Octagonal Section,"* by G. K. Batchelor, M.Sc.

*Pamphlet No. 109.—“Studies on the Physiology and Toxicology of Blowflies. 8. Rate of Ammonia Production by Larvae of *Lucilia cuprina* and its Distribution in this Insect. 9. The Enzymes Responsible for Ammonia Production by Larvae of *Lucilia cuprina*,”* by F. G. Lennox, M.Sc., A.I.C.

*Pamphlet No. 110.—“The Main Virus Diseases of the Potato in Victoria,”* by J. G. Bald, M.Agr.Sc., Ph.D., and A. T. Pugsley, B.Agr.Sc.

*Pamphlet No. 111.—“The Biology and Cultivation of Oysters in Australia. II. A Note on the Calcium Content of Some East Australian Waters. III. Biochemistry of the Proximate Constituents,”* by George Humphrey, M.Sc.

COMMONWEALTH



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